Exhibit 56

Perineal Talc Use and Ovarian Cancer

A Systematic Review and Meta-Analysis

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Background: It has been posited that there is an association between perineal talc use and the incidence of ovarian cancer. To date, this has only been explored in observational studies.

Objectives: To perform a meta-analysis to evaluate the association between perineal talc use and risk of ovarian cancer.

Methods: Studies were identified using six electronic databases. Observational studies involving at least 50 cases of ovarian cancer were eligible for inclusion. We analyzed the association between ovarian cancer, including specific types, and any perineal talc use, long-term (>10 years) use, total lifetime applications, and use on diaphragms or sanitary napkins. A subgroup analysis was performed, stratifying by study design and population.

Results: We identified 24 case–control (13,421 cases) and three cohort studies (890 cases, 181,860 person-years). Any perineal talc use was associated with increased risk of ovarian cancer (OR = 1.31; 95% CI = 1.24, 1.39). More than 3600 lifetime applications (OR = 1.42; 95% CI = 1.25, 1.61) were slightly more associated with ovarian cancer than <3600 (OR = 1.32; 95% CI = 1.15, 1.50). An association with ever use of talc was found in case–control studies (OR = 1.35; 95% CI = 1.27, 1.43), but not cohort studies (OR = 1.06; 95% CI = 0.90, 1.25). However, cohort studies found an association between talc use and invasive serous type ovarian cancer (OR = 1.25; 95% CI = 1.01, 1.55). We found an increased risk of serous and endometrioid, but not mucinous or clear cell subtypes.

Conclusions: In general, there is a consistent association between perineal talc use and ovarian cancer. Some variation in the magnitude of the effect was found when considering study design and ovarian cancer subtype.

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Ovarian cancer is the gynecologic cancer associated with the highest mortality in the United States, in 2012 being the fifth highest cause of cancer death in women with 14,404 deaths in that country.[1] The National Cancer Institute's Surveillance, Epidemiology, and End Results Program (SEER) predicts that in the United States, in 2016, there will be 22,280 incidences of newly diagnosed ovarian cancer, and 14,240 deaths caused by ovarian cancer based on age-adjusted data from 2009 to 2013.[2] The 5-year survival statistics for ovarian cancer are poor, largely because patients usually present with advanced disease, which is less amenable to curative therapy.[3] SEER estimates that only 15% of patients present with disease localized to the ovary, which contributes to a 5-year survival of 46.2%.[2] It is imperative to develop public health programs, which either reduce the incidence of ovarian cancer or detect it at an earlier stage, to reduce the burden of this disease.

Routine pelvic examinations, transvaginal ultrasonography, and tumor markers have been trialed as potential screening tools for ovarian cancer, but are limited in their usefulness. The cancer marker cancer antigen 125 (CA-125, also known as mucin 16) has been found to be elevated in 80% of all ovarian carcinomas, but this falls to 50% in women in which the cancer is localized only to the ovary, where it is most amenable to treatment.[4] As CA-125 has a low sensitivity and limited specificity, it is not recommended as a screening test for women without clinical symptoms.[5] Ultrasound has a reasonable sensitivity but poor specificity and positive predictive value, particularly as it is poor at distinguishing between benign and malignant masses.[6] While the search for an effective screening regimen for ovarian cancer continues, the importance of primary prevention becomes paramount.

Talcum powder is made of talc, a hydrated magnesium silicate, and is used to absorb moisture on the body. Some women choose to dust talc on the perineum, or apply it to diaphragms or sanitary napkins, to reduce friction, keep the skin dry, reduce odor, and prevent rashes. The potential association between perineal talc use and ovarian cancer has been discussed for decades. The first investigation of this association was performed by Cramer et al[7] in 1982, when the investigators found a relative risk of 1.92 (95% CI = 1.27, 2.89) for ovarian cancer when women either dusted the perineum with talc powder or used it on sanitary napkins. Since this time, there has been substantial interest in and research into this association.

In the present context, the association between talc use and ovarian cancer takes on considerable relevance, as the pharmaceutical and consumer products company Johnson & Johnson has recently had damages levied to the total of US$717 million against them in five law suits. In these cases, juries decided that the use of talcum powder caused or contributed to the development of the plaintiff's ovarian cancer. The evidence for the association between perineal talc use and ovarian cancer is based on the body of knowledge from observational studies, and most of these have been retrospective case–control studies prone to recall bias. Hence, while perineal talc use has not been shown to be safe, in a similar regard, a certain causal link between talc use and ovarian cancer has not yet been established.[8,9]

In 2013, a pooled analysis was performed for eight population-based case–control studies, and found a modest increased risk (OR = 1.24) of ovarian carcinoma associated with perineal talc use.[10] In 2007, a meta-analysis was performed of nine observational studies; however, this study only examined the use of talc on contraceptive diaphragms.[11] The overall finding of this meta-analysis was that the use of talc on contraceptive diaphragms was not associated with ovarian cancer. Meta-analyses have been performed on this subject before; however, the most recent was in 2008,[9] and since this time, the results of a number of large case–control studies and two cohort studies[12,13] have been published. Hence, there is a need to update the literature, particularly considering pending litigation against Johnson & Johnson by other claimants, and Johnson & Johnson's potential plans to appeal the previous decisions. Furthermore, producers of talcum powder products continue to sell these products without any warning labels regarding perineal use and potential associations with ovarian cancer. Hence, there is a need for clarification, to allow women to be adequately informed of the risk of use of these products, possibly preventing future harm.

This paper aims to review the literature and provide an overall risk estimate for the association between perineal talc use and ovarian carcinoma. We will also perform subgroup analyses by the method of talc application, the duration of talc use, the total number of perineal talc applications, and the type of ovarian cancer developed to further elucidate the relationship between talc use and ovarian carcinoma.

METHODS

Study Protocol

We followed the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines.[14] R.P. performed a systematic search of the databases MEDLINE (from 1950), PubMed (from 1946), Embase (from 1949), the Cumulative Index to Nursing and Allied Health Literature (CINAHL), LILACS, and the Cochrane Central Register of Controlled Trials through 22 August 2017 to identify relevant articles. The search used the terms ("talc" OR "talcum powder") AND ("ovarian cancer" OR "ovarian carcinoma"), which were searched as text word and as exploded medical subject headings where possible. We also searched the reference lists of relevant articles for appropriate studies. No language restrictions were used in either the search or study selection. We did not search for unpublished literature.

Study Selection

We included studies that met the following inclusion criteria: (1) the study investigated the perineal use of talc in relation to risk of development of ovarian cancer; (2) the study reported adverse events as an odds ratio (OR), or the data were presented such that an OR could be calculated; (3) the 95% confidence interval (CI) was reported, or the data were presented such that the CI could be calculated; and (4) the study involved a minimum of 50 cases. We excluded studies that did not meet the inclusion criteria.

Data Extraction

One of us (R.P.) performed data extraction using a standardized data extraction form, collecting information on the publication year, study design, number of cases, number of controls, total sample size, population type, country, mean age, number of adjusted variables, the risk estimates or data used to calculate the risk estimates, CIs or data used to calculate CIs, and the type of ovarian cancer. R.P. assessed the quality of the studies using the Newcastle-Ottawa Scale (NOS); however, no studies were excluded on the basis of NOS score.[15] Authors were not contacted for missing data. Adjusted ratios were extracted in preference to nonadjusted ratios; however, where ratios were not provided, R.P. calculated unadjusted ORs and CIs.

Statistical Analysis

One of us (G.D.E.) calculated pooled ORs and 95% CIs for the effect of any perineal talc use with all ovarian cancers using a random effects model.[16] Analyses were also performed based on the method of administration (diaphragm, sanitary napkins), duration of use, and type of ovarian cancer developed (all mucinous, mucinous invasive, mucinous borderline, all serous, serous invasive, serous borderline, endometrioid, clear cell). For long-term talc use, we extracted the odds ratio for the group with the longest duration of talc exposure compared with controls, provided that group used talc for a minimum duration of 10 years. For overall lifetime talc applications, groups within each study were divided into either <3600 lifetime applications, equivalent to less than approximately 10 years of daily use, or >3600 applications. Where a group from a study did not completely fit into this dichotomy, we placed it into the category it most closely fit. Details on the categorization of individual groups are available in eTable 1 (http://links.lww.com/EDE/B261). Odds ratios were pooled for invasive serous, invasive mucinous, borderline serous, and borderline mucinous tumors individually. However, as many studies reported only all mucinous or all serous in a single

group, we also ran analyses for risk associated with all mucinous and all serous tumors. Where a study reported separately as borderline and serous, both odds ratios were included separately in the meta-analysis, to ensure all available data were considered.

We tested heterogeneity with Cochran's Q statistic, with $P < 0.10$ indicating heterogeneity, and quantified the degree of heterogeneity using the I^2 statistic, which represents the percentage of the total variability across studies which is due to heterogeneity. I^2 values of 25%, 50%, and 75% corresponded to low, moderate, and high degrees of heterogeneity, respectively.[17] We quantified publication bias using the Egger's regression model,[18] with the effect of bias assessed using the fail-safe number method. The fail-safe number was the number of studies that we would need to have missed for our observed result to be nullified to statistical nonsignificance at the $P < 0.05$ level. Publication bias is generally regarded as a concern if the fail-safe number is less than 5n + 10, with n being the number of studies included in the meta-analysis.[19] All analyses were performed with Comprehensive Meta-analysis (version 3·0; Biostat, Englewood, NJ; 2014).

RESULTS

Study Characteristics

We performed a broad literature search of electronic databases, identifying 363 citations for review (Figure 1). Initially, 318 studies were discarded, with many being narrative reviews, duplicates, animal studies, opinion pieces, editorials, or otherwise irrelevant. Forty-five citations were selected for full-text review. Of these, three were excluded due to being associated with endometrial rather than ovarian cancer, two were meta-analyses, five were duplications of data from the same study, one involved non-perineal application of talc, and seven were otherwise irrelevant. No studies were excluded for failing to report an odds ratio or for not providing the necessary raw data from which an odds ratio could be provided. Some studies provided only the raw data, i.e., the number of cases and controls with and without perineal talc use. This allowed an unadjusted odds ratio to be calculated, which was then included in the analysis. Overall, 27 studies were selected. Note that Wu et al[33] (2015) include results from Wu et al[36] (2009); however, only Wu et al[36] (2009) reported on non-perineal talc use, total lifetime applications, and long-term talc use. Hence data were extracted from Wu et al[33] (2015) for the "any perineal use" outcome, and from Wu et al[36] (2009) for the three other outcomes previously mentioned. Hence, while 27 studies were included in the analysis, only 26 were included in the any perineal use analysis. Three studies were cohort studies, including 890 cases and 181,860 person-years.[12,13,20] The remaining 26 studies were case–control studies, with a total of 13,421 cases and 19,314 controls. The case–control studies are described in eTable 1 (http://links.lww.com/EDE/B261), while the cohort studies are described in eTable 2 (http://links.lww.com/EDE/B261). In total, studies involving 14,311 cases of ovarian cancer were included in this review.

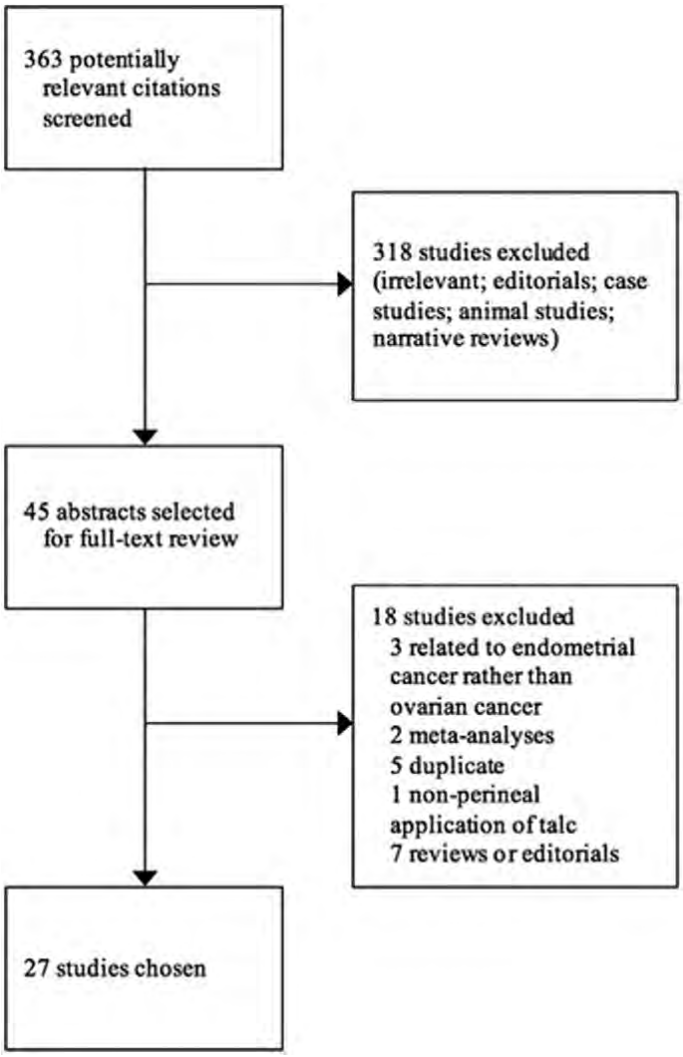

FIGURE 1. PRISMA flowchart for literature search and study selection.

The quality of the studies was assessed using the Newcastle-Ottawa Scale (NOS), which involves separate assessment tools for both case–control and cohort studies.[15] The highest score awarded was 8/10, and the lowest was 5/10. The mean score was 7.0. Almost all studies lost points because the exposure to talc was ascertained through self-report rather than an independently verified source, and because the interviewer was not blinded to cases and controls. Many studies also failed to specifically describe that their chosen controls did not have a personal history of previous ovarian cancer. It may be the case that this was done, but not reported in the study methods. Generally, case ascertainment and matching controls based on age and other factors, often geographical location or ethnicity, were well performed in the reviewed studies. The breakdown of individual study scores is included in Tables 1 and 2. Overall, the quality of studies included in

TABLE 1. Summary of Pooled Effect Sizes for Examined Outcome Variables

	No. Studies	Effect Size	Heterogeneity		Publication Bias
		OR (95% CI)	I^2	*P*	*P*
Method of talc use					
Any perineal	26	1.31 (1.24, 1.39)	10.52	0.31	0.09
Any non-perineal	5	1.24 (1.01, 1.51)	66.84	0.02	0.86
Diaphragm	8	0.84 (0.68, 1.05)	14.76	0.31	0.64
Sanitary napkins	12	1.15 (0.94, 1.41)	43.82	0.05	0.17
Length of talc use					
Long-term use (>10 years)	12	1.25 (1.10, 1.43)	45.11	0.04	0.31
<3600 total applications	5	1.32 (1.15, 1.50)	1.83	0.41	0.20
>3600 total applications	5	1.42 (1.25, 1.61)	12.59	0.33	0.40
Type of ovarian cancer					
All serous	10	1.32 (1.22, 1.43)	0.00	0.75	0.44
Serous invasive	5	1.32 (1.13, 1.54)	25.10	0.25	0.75
Serous borderline	3	1.39 (1.09, 1.78)	0.00	0.94	0.83
All mucinous	9	1.12 (0.94, 1.33)	5.79	0.39	0.79
Mucinous invasive	2	1.34 (0.48, 3.79)	69.39	0.07	NA[a]
Mucinous borderline	3	1.18 (0.76, 1.81)	34.07	0.22	0.96
Endometrioid	8	1.35 (1.14, 1.60)	0.00	0.61	0.78
Clear cell	3	1.02 (0.75, 1.39)	0.00	0.78	0.22

[a]NA = not applicable; no publication bias ... result available when there are fewer than three studies in the analysis.

this review was reasonably high. No studies were excluded from the review based on NOS score.

All studies reported at least an odds ratio for any perineal use of talc and its association with ovarian cancer. As previously described, Wu et al[36] (2009) was not included in this analysis to prevent duplication of data. Five studies reported on only non-perineal exposure. Additionally, eight studies provided data for use of talc on a diaphragm, and 12 for sanitary napkins. Twelve studies provided an odds ratio for long-term talc use and its association with ovarian cancer; however, the chosen threshold for long term was variable, from more than 10 years to more than 37.4 years. Five studies reported on the total number of talc applications. It was frequently necessary to report different groups from a single study separately to perform the meta-analysis of this outcome, with the groupings being described specifically in eTable 1 (http://links.lww.com/EDE/B261). Ten studies reported odds ratios for all serous ovarian cancers, five reported for serous invasive cancers, and three reported for serous borderline cancers. Similarly, nine reported for all mucinous cancers, two for mucinous invasive, and three for mucinous borderline. Eight studies reported odds ratios for endometrioid ovarian cancer, and three reported for clear cell ovarian cancer.

Quantitative Data Synthesis

The results of the initial pooling of data from all studies are summarized in Table 1. Pooling of data revealed an increased risk of ovarian cancer associated with any perineal use of talc (Figure 2A; OR = 1.31; 95% CI = 1.24, 1.39). Use of talc long term (>10 years) was also associated with an increased ovarian cancer risk (Figure 2B; OR = 1.25; 95% CI = 1.10, 1.43). Both <3600 total lifetime applications (OR = 1.32; 95% CI = 1.15, 1.50) and >3600 lifetime applications (OR = 1.42; 95% CI = 1.25, 1.61) of talc were associated with an increased risk of ovarian cancer, with a slightly higher risk in the group with greater usage. Talc use on diaphragms or on sanitary napkins was not individually associated with increased risk of ovarian cancer. Any perineal talc use was associated with any serous (Figure 2C; OR = 1.32; 95% CI = 1.22, 1.43), serous invasive (OR = 1.32; 95% CI = 1.13, 1.54), serous borderline (OR = 1.39; 95% CI = 1.09, 1.78), and endometrioid (Figure 2D; OR = 1.35; 95% CI = 1.14, 1.60) subtypes of ovarian cancer, but not the other subtypes.

We performed a subgroup analysis stratifying by study design. It is important to note that there were only three cohort studies, each of which did not report on all the assessed associations. For any perineal talc use, only case–control studies showed an association with ovarian cancer (Figure 2A; OR = 1.35; 95% CI = 1.27, 1.43), while no association was noted for cohort studies (OR = 1.06; 95% CI = 0.90, 1.25). For the other associations assessed, the results are reported in Table 2. In cohort studies, the only association found was between perineal talc use and the incidence of serous invasive cancer subtypes (OR = 1.25; 95% CI = 1.01, 1.55). For borderline serous, borderline mucinous, invasive mucinous, and clear cell ovarian cancer subtypes, no cohort studies provided data for the association and hence the odds ratios reported in eTable 2 (http://links.lww.com/EDE/B261) are derived entirely from case–control studies. The only outcome reported in all three cohort studies was any perineal talc use; hence the available data from prospective studies were limited.

A subgroup analysis related to study population setting, i.e., in the hospital or in the general population, was performed for any perineal talc application. Generally, hospital-based studies were older (pre-2000) than the community-based studies. There were seven hospital-based studies, all of which were case–control studies. There were 20 population-based studies, including 17 case–control studies and all three cohort studies. There was no difference between the pooled results for hospital- and population-based studies (OR = 1.22 vs. 1.33), respectively.

There was heterogeneity in the analysis of non-perineal applications of talc (I^2 = 66.84; P = 0.02). There was no heterogeneity for any of the other outcome measures in either the meta-analysis of all available studies or the subgroup analyses. There was no publication bias in the meta-analysis of any genital talc exposure and ovarian cancer, which included all the studies in the review, except Wu et al[36] (2009) (Figure 3; P = 0.09). The result for publication bias for each of the individual analyses is included in Table 1.

TABLE 2. Summary of Pooled Effect Sizes in Subgroup Analysis by Study Design

	Case–Control Studies (n = 24)				Cohort Studies (n = 3)			
		Effect Size	Heterogeneity			Effect Size	Heterogeneity	
	No. Studies	OR (95% CI)	I^2	*P*	No. Studies	OR (95% CI)	I^2	*P*
Method of talc use								
Any perineal use	23	1.35 (1.27, 1.43)	0.00	0.77	3	1.06 (0.90, 1.25)	18.89	0.29
Non-perineal use	5	1.24 (1.01, 1.51)	66.84	0.02	0	NA	NA	NA
Diaphragm	7	0.81 (0.61, 1.08)	21.92	0.26	1	0.92 (0.68, 1.24)	0.00	1.00
Sanitary napkin	10	1.27 (0.98, 1.65)	40.49	0.09	2	0.93 (0.77, 1.13)	0.00	0.77
Length of talc use								
Long-term use	11	1.29 (1.13, 1.47)	40.53	0.08	1	0.98 (0.75, 1.29)	0.00	1.00
<3600 total applications	5	1.32 (1.15, 1.50)	1.83	0.41	0	NA	NA	NA
>3600 total applications	5	1.42 (1.25, 1.61)	12.59	0.33	0	NA	NA	NA
Type of ovarian cancer								
All serous	12	1.34 (1.23, 1.47)	0.00	0.71	2	1.19 (0.97, 1.47)	0.00	0.61
Serous invasive	3	1.36 (1.05, 1.75)	47.96	0.15	2	1.25 (1.01, 1.55)	0.00	0.33
Serous borderline	3	1.39 (1.09, 1.78)	0.00	0.94	0	NA	NA	NA
All mucinous	9	1.15 (0.93, 1.41)	21.03	0.26	2	0.96 (0.61, 1.53)	0.00	0.84
Mucinous invasive	2	1.34 (0.48, 3.79)	69.39	0.07	0	NA	NA	NA
Mucinous borderline	3	1.18 (0.76, 1.81)	34.07	0.21	0	NA	NA	NA
Endometrioid	6	1.39 (1.16, 1.66)	0.00	0.52	2	1.09 (0.66, 1.80)	0.00	0.48
Clear cell	3	1.02 (0.75, 1.39)	0.00	0.78	0	NA	NA	NA

NA = not applicable; no cohort studies reported on the relevant associations.

DISCUSSION

The present meta-analysis reports a positive association between perineal talc use and ovarian cancer, specifically of the serous and endometrioid histologic subtypes. The mechanism by which perineal talc use may increase the risk of ovarian cancer is uncertain. It has been previously proposed that talc, as a foreign body, may ascend from the vagina through to the uterine tubes and instigate a chronic inflammatory response, which may predispose to the development of ovarian cancer. It is argued that cellular injury, oxidative stress, and local increase in inflammatory mediators such as cytokines and prostaglandins may be mutagenic and hence promote carcinogenesis.[21] In support of this hypothesis, it has been found that hysterectomy or bilateral tubal ligation, in which ovarian exposure to inflammatory mediators would be significantly curtailed, is associated with a reduced risk of ovarian cancer.[22–24] However, the use of non-steroidal anti-inflammatory drugs (NSAIDs) is not inversely associated with the incidence of ovarian cancer, as may be expected if the etiology was related to chronic inflammation.[25,26] It has also been found that human epithelial ovarian cells have an unusually low expression of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), which would reduce their sensitivity to the action of NSAIDs.[27] The potential mechanism by which genital talc is associated with an increased risk of ovarian cancer hence remains unclear.

An important finding of this study is that talc use appears to be associated with increased risk of serous ovarian cancer, of both invasive and borderline types, and not with mucinous ovarian cancer. Additionally, endometrioid ovarian cancers but not clear cell cancers were significantly associated with perineal talc use. Intriguingly, a meta-analysis examining the effects of tubal ligation of ovarian cancer risk found a reduced risk of the same subtypes of ovarian cancer as mentioned here: serous and endometrioid, but not mucinous.[24] If chronic inflammation due to ascending foreign bodies is indeed the mechanism by which talc use is associated with increased ovarian cancer risk, then these results fit the picture. The results for non-perineal application of talc were still positive but of lower magnitude, supporting the hypothesis of ascending foreign bodies causing chronic inflammation. It is plausible that non-perineal application of talc may cause increased risk through, e.g., the respiratory tract. Unfortunately, the evidence remains insufficient to understand the mechanism with any reasonable certainty.

We also found a slightly greater increased risk of ovarian cancer with >3600 lifetime applications compared with those with <3600 lifetime applications. The number of lifetime applications is a more valid measure of the patient's exposure to perineal talc than either duration or frequency of use alone. This finding also supports the chronic inflammatory hypothesis, as repeated exposure would induce a longer period of chronic inflammation, and therefore should increase the predisposition to the development of ovarian cancer. It is notable that these data were only available from

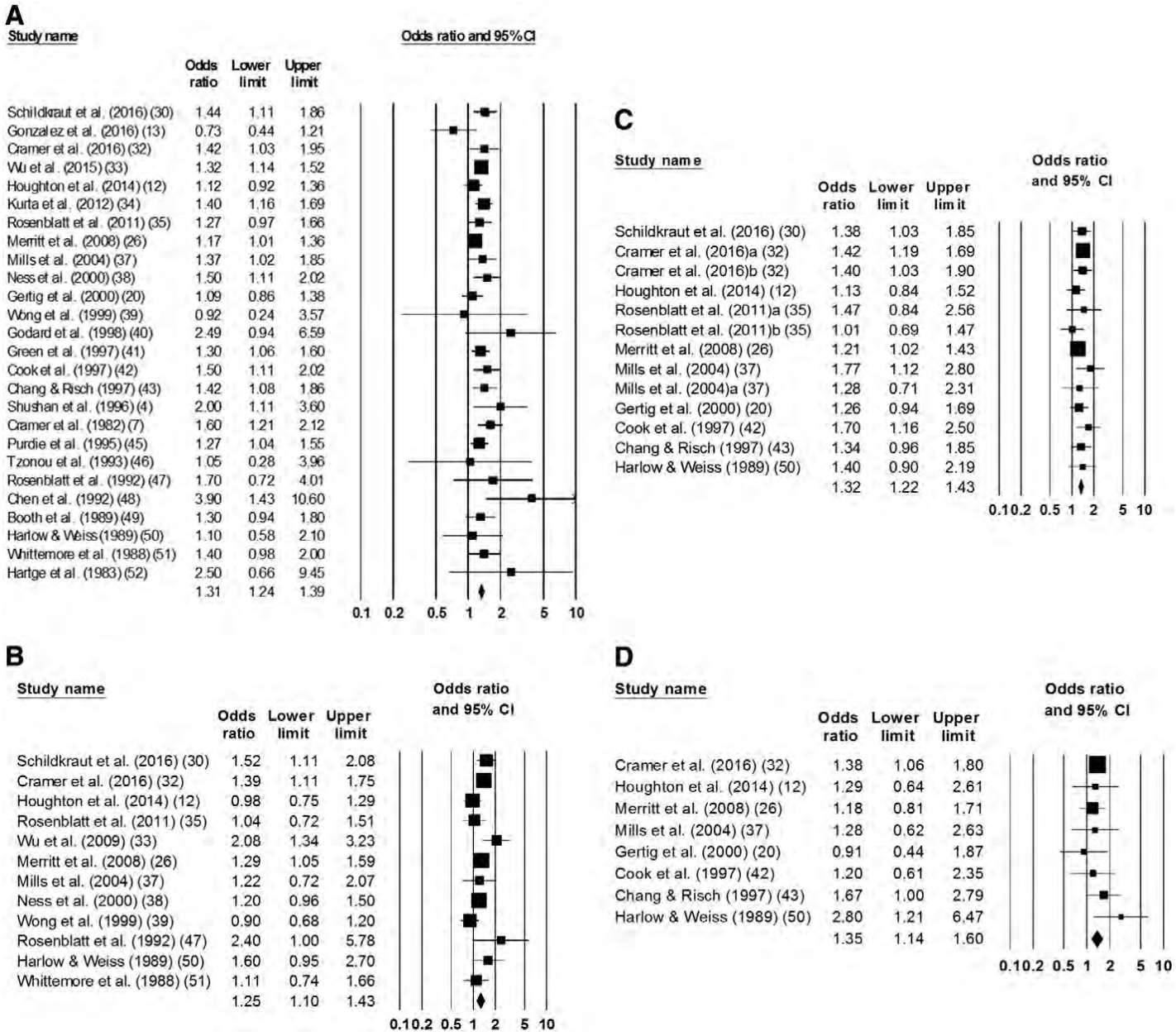

FIGURE 2. A, Any perineal talc use is associated with an increased risk of any ovarian cancer (OR = 1.31; 95% CI = 1.24, 1.39). B, Long-term perineal talc use (>10 years use) is associated with an increased risk of any ovarian cancer, but of a lower magnitude than any perineal use (OR = 1.25; 95% CI = 1.10, 1.43). C, Any perineal talc use is associated with an increased risk of serous ovarian cancers (OR = 1.32; 95% CI = 1.22, 1.43). D, Any perineal talc use is associated with an increased risk of endometrioid type ovarian cancers (OR = 1.35; 95% CI = 1.14, 1.60).

case–control studies, as the three cohort studies did not sufficiently record duration and frequency of use to be included in the analysis. This retrospective finding is therefore prone to recall bias.

This meta-analysis had several strengths. None of the analyses in this review had statistically significant heterogeneity, except for non-perineal application, which indicates consistency in the direction and magnitude of the effect size between individual studies, and strengthening the reliability of the pooled effect sizes. Another strength of this review is the large number of overall cases (n = 14,311), improving the power of the meta-analysis to detect a relatively small effect size, as occurred in this case. Another strength of this review is that the included studies were of relatively high quality as assessed through the NOS, reducing the potential for bias in the conclusions drawn. The NOS revealed that the most common limitations of the included case–control studies were the failure to blind interviewers to case–control status of subjects in the interview, and reliance on memory and self-report for collection of data on perineal talc use.

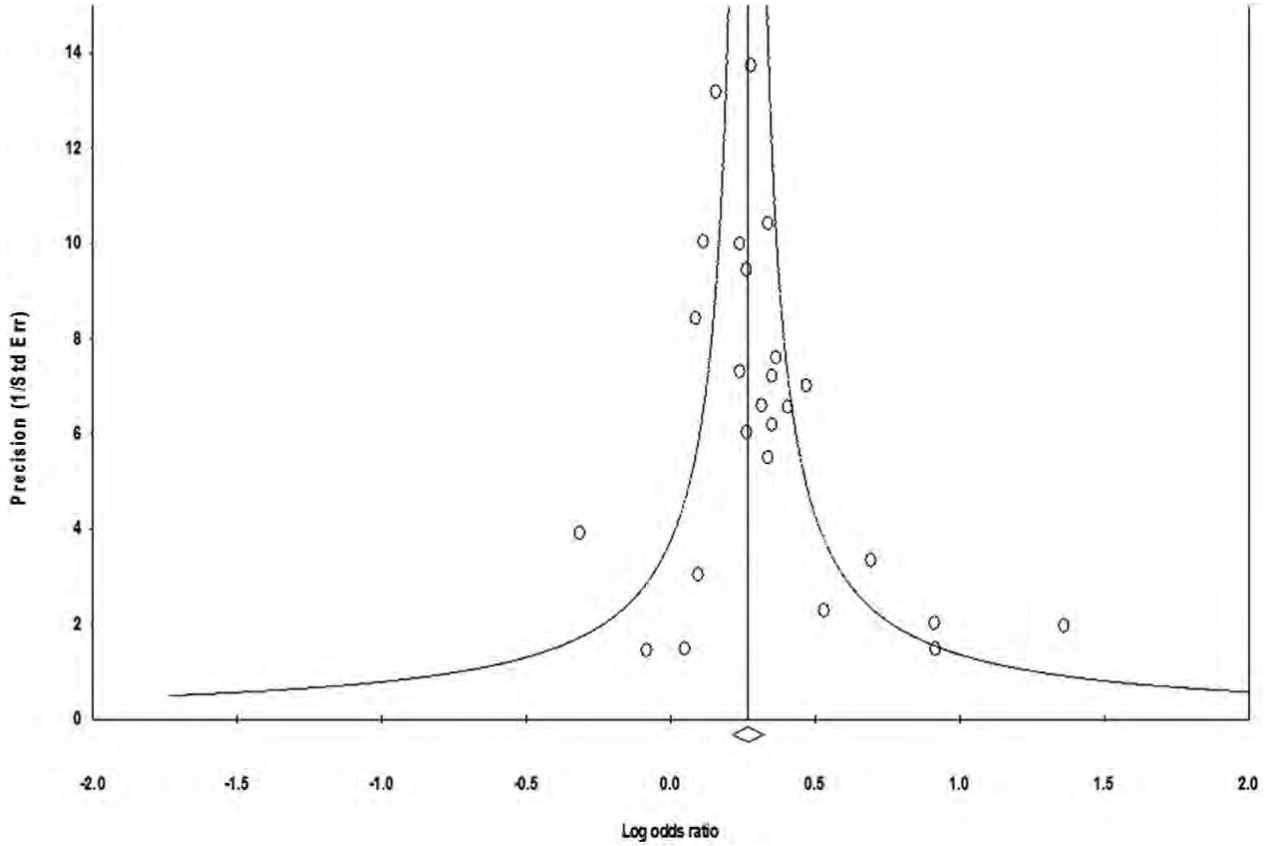

FIGURE 3. Funnel plot for the meta-analysis of studies examining any perineal talc use and risk of ovarian cancer ($P = 0.09$).

A limitation of this study is that it pools nonrandomized studies, primarily case–control studies. The retrospective nature of case–control studies introduces the potential for recall bias. In this case, it is entirely possible that patients with ovarian cancer may be more aware of their previous talc use and hence be more likely to report higher past use. It is possible to attempt to overcome this by blinding the participants to the nature of the study, usually by asking spurious questions; however, the effectiveness of this approach may be limited.[28] Many of the studies in this review recorded data about talc use as part of a more extensive questionnaire focused on other associations, which may reduce the potential for recall bias. However, since the initiation of lawsuits in 2014, there has been extensive media coverage regarding this association, and the potential for recall bias in case–control studies conducted since then may be exacerbated.

Cohort studies are useful in that they are prospective; however, the low incidence of ovarian cancer results in relatively small number of cases even in large cohorts, as seen in the three cohort studies included in this review.[29] Considering potential exposure misclassification issues in case–control studies, the effect for any perineal talc use was very weak in a small number of cohort studies. However, an association between talc use and serous invasive ovarian cancer was found.

Of the studies in this review, case–control studies achieved much large number of cases, in some instances in excess of 2000 cases and a similar number of age-matched controls, which provide greater statistical power for the detection of an effect size of small magnitude. Hence while case–control studies are low-level evidence, they have been preferred in the investigation of the association between talc use and ovarian cancer. They also have the important advantage of not requiring 15 or more years of follow-up, as is necessary for a cohort study to sufficient detect cases of ovarian cancer relative to certain exposures. One potential way to overcome this limitation in future studies is to ensure that talc use is always included in questionnaires of any cohort studies investigating ovarian cancer. It is important not only that talc use be investigated but also the precise location, duration, and frequency of use. As it stands, a meta-analysis of observational studies such as the present study provides the highest level of evidence practically feasible for this research question.

CONCLUSIONS

The results of this review indicate that perineal talc use is associated with a 24%–39% increased risk of ovarian cancer. While the results of case–control studies are prone to recall bias, especially with intense media attention following the commencement of litigation in 2014, the confirmation of an association in cohort studies between perineal talc use and serous invasive ovarian cancer is suggestive of a causal association. Additional epidemiologic evidence from prospective

studies with attention to effects within ovarian cancer subtype is warranted. There is a substantial need for further research on a potential mechanism by which ovarian cancer may be caused by talc, as this will allow a causal relationship to be established or rejected with more certainty. However, particularly because of the dearth of screening tests available for this high-mortality cancer, it is important that research into this association continue as it is a potential avenue for cancer prevention.

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Exhibit 57

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PERINEAL TALC EXPOSURE AND EPITHELIAL OVARIAN CANCER RISK IN THE CENTRAL VALLEY OF CALIFORNIA

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Perineal talc use has been suggested as a possible risk factor for ovarian cancer based on its structural similarity to asbestos, a known human carcinogen. A population-based epidemiologic case-control study of epithelial ovarian cancer (EOC) was conducted in 22 counties of Central California that comprise the reporting area for 2 regional cancer registries. Telephone interviews were conducted with 256 cases diagnosed in the years 2000 2001 and 1,122 controls frequency-matched on age and ethnicity. The interview obtained information on demographic factors, menstrual and reproductive experience, exogenous hormone use, surgical history and family history of cancer. Questions on perineal talc use included frequency of use, duration of use and specific years when talc was used. Multivariate-adjusted odds ratio (OR) and 95% confidence intervals (CI) were derived from unconditional logistic regression. The OR for ever use of talc was 1.37 (CI 1.02 1.85) compared to never users. However, no dose response association was found. Tubal ligation (TL) modified the effect of talc on EOC such that women with TL had an OR of 0.88 (CI 0.46 1.68) associated with perineal talc use, whereas women with no TL had an OR of 1.54 (CI 1.10 2.16). Talc use and EOC risk was highest in women with serous invasive tumors (OR 1.77; CI 1.12 2.81). This study provides some support for the hypothesis that perineal talc use is associated with an increased risk of EOC.

Key words: *epidemiology; gynecologic cancer; risk factors*

Interest in the relationship between talcum powder and ovarian cancer risk is based on certain physical properties of talcum powder, including the fact that talc is mineralogically similar to asbestos and that talcum powder manufactured before 1973 may have been contaminated with asbestos.[1] In animal studies, talc and other similar substances have been demonstrated to migrate from the vagina through the peritoneal cavity to the ovaries.[2] Henderson *et al*.[3] also observed that particles with the appearance of talc were more prevalent in ovarian tumors than in normal ovarian tissue. Several epidemiologic studies have investigated perineal use of talcum powder as a potential risk factor for ovarian cancer and most have found elevations in risk, although there has been a large range in the risk estimates, from 1.1 to 3.9.[4] Collectively, these studies point to a possible etiologic role of talc in ovarian cancer via an inflammatory process at the site of the ovarian epithelium,[5] although recall bias may play a role in retrospective studies.[4] Inflammation produces oxidants that are thought to damage DNA and Ames *et al*.[6] argue that damage to tumor suppressor genes caused by the inflammatory process leads to carcinogenesis. Chronic inflammation may also result in deregulated cytokine production, which may result in altered cell growth, inhibition of apoptosis and changes in differentiation.[7]

Cramer *et al*.[8] proposed 2 mechanisms that might explain talc carcinogenesis. Talc may stimulate the entrapment of the ovarian surface epithelium, thus mimicking what occurs during ovulation and posing a risk similar to that proposed by incessant ovulation.[9] Alternatively, if talc is present at the time of ovulation, it may become incorporated into the inclusion cyst. It has been suggested that foreign-body exposure may result in granulomas[10] and that pure talc may induce granulomas in open wounds.[11] Granulomas are also associated with persistent acute inflammatory responses.[12]

The role of cornstarch powder on ovarian cancer risk has also been evaluated in epidemiologic research and a recent review concluded that there is no association between this type of powder and increased risk of ovarian cancer.[13] The conclusion was based on a total of 4 case-control studies that elicited information on use of cornstarch in perineal dusting, in which the average odds ratio was 0.62. However, there were only a total of 20 cases of ovarian cancer combined in those studies and 51 control subjects. Cornstarch is also not thought to exert the same toxicologic reaction in human tissue as does talc.[13]

MATERIAL AND METHODS

A population-based epidemiologic case-control study of epithelial ovarian cancer (EOC) was conducted in 22 counties of Central California that comprise the reporting area for 2 regional cancer registries. Geographically, these counties make up the majority of the Central Valley of California, which is the poorest area of the state, with many residents living below the poverty level.[14] Demographically, the Valley is a very ethnically diverse area in which many counties are over 40% Hispanic. Two population-based cancer registries have monitored cancer incidence in the Central Valley of California continuously since 1988: the Cancer Registry of Central California (CRCC) in Fresno and the Cancer Surveillance Program (CSP), Region 3, in Sacramento.[15,16] All newly diagnosed histologically confirmed EOC patients were available for inclusion in this study for the years 2000 and 2001.

Cases were women-identified via a rapid case ascertainment (RCA) procedure as having been diagnosed with EOC (malignant neoplasms of the ovary, ICD-O 3 C56.9) living in the Central Valley during a 24-month period from 1 January 2000 through 31 December 2001. Tumors were designated as borderline if the behavior code was designated as 1, or if the pathology report described the tumor as borderline, low malignant potential, or atypically proliferating.[17] The borderline classification was limited to serous and mucinous cell types because ICD-O 3 has no morphology code for the borderline classification in the other subtypes and because serous and mucinous tumors make up the majority of borderline tumors.[18] All other tumors were classified as invasive.

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Histologic subtypes were identified by pathologic report or by ICD-O 3 morphology codes. The histologic subtypes included were serous, mucinous, endometrioid, clear cell and other epithelial/unclassified. The latter category included unspecified adenocarcinomas as well as undifferentiated tumors in which a cell type could not be classified histologically. All newly diagnosed EOCs of epithelial origin were identified via RCA methods in which hospital tumor registrars were asked to provide listings of newly diagnosed EOCs within 1 month of diagnosis. A board-certified pathologist reviewed the pathology reports of a sample of cases. Physician consent was obtained by mailing the physician of record a letter and informing him/her that an interview with the patient was planned. If the physician did not respond within a 3-week period, passive consent was assumed. The control group consisted of women 18 years or older selected by random digit dialing (RDD) techniques who were residents of the area, had not been diagnosed with EOC and had at least one intact ovary at the time of the interview. Controls were frequency matched to cases on age and race/ethnicity. The overall data collection period covered a 2-year period, with each respondent being interviewed only once during this period by telephone. Interviews were conducted with both cases and controls on a monthly basis throughout the 2-year period.

All cases and controls were approached via an introductory letter that included a prompt list that described topics the interview questions would address. The Institutional Review Board at the Public Health Institute approved the study protocol. For both case and control groups, letters and prompt lists were sent in either English or Spanish on the letterhead of the principal investigator. Telephone interviews with both case and control respondents were conducted by female professional, trained telephone interviewers in either English or Spanish as preferred by the respondent.

The interview obtained information on demographic factors as well as information pertinent to the respondent s menstrual and reproductive experience, use of exogenous hormones, gynecologic surgical history and family history of cancer. Four questions were asked in regard to the use of talcum powder, including adult use in the genital area, calendar year(s) of use, frequency of use (*i.e.*, daily, several times a week) and total duration of use. The last 2 questions were used to create a variable reflecting—cumulative use by combining frequency (categorically weighted 0 3) and duration (in months) of use.

Age-adjusted odds ratios were calculated using the Mantel-Haenszel method.[19] Multivariate adjusted odds ratios were calculated using unconditional logistic regression.[20] Initially, multivariate models were constructed to include age as a continuous variable and race/ethnicity, duration of use of oral contraceptives, duration of breast-feeding, history of breast or EOC in a first-degree relative, pregnancy history, parity, body mass index (BMI), hysterectomy, tubal ligation and duration of hormone replacement therapy use as categorical variables. However, the Hosmer-Lemshow goodness-of-fit tests revealed that after terms for duration of oral contraceptive use and duration of breast-feeding were added to the models, fit was not improved by the addition of the other variables listed above. Nor were the estimated odds ratios altered by the addition of the several variables listed above. Therefore, in the interest of parsimony, the final models chosen for the analysis included terms for age, race/ethnicity, oral contraceptive use and breast-feeding. Interaction was assessed by comparing stratum-specific odds ratios. If the stratum-specific odds ratios differed by more than 100%, interaction was also assessed by including first-order cross product terms into the logistic model and examining the significance of the interaction coefficient. Tests for trend were conducted for variables that were ordinal in nature by recoding the categories into continuous form and evaluating the Wald statistic associated with the resulting coefficient. Confounding was assessed by examining the differences in the crude, age-adjusted and multivariate-adjusted odds ratios.

RESULTS

The regional cancer registries initially identified a total of 652 cases of confirmed epithelial ovarian cases residing in the 22 county study area diagnosed between 1 January 2000 and 31 December 2001. Seventeen cases were excluded due to speaking a language other than English or Spanish or due to hearing/speech impairment, resulting in 635 cases that met the study criteria. Seventy-six cases died prior to research contact and physicians refused permission to contact for 10 cases. Forty-one cases were too ill to participate in the study and 119 were not contacted due to incorrect telephone numbers or no answer after repeated efforts. Of the remaining 389 cases, 133 refused to participate, resulting in 256 completed interviews. Therefore, the response fraction was 40% among all cases identified. There were no significant differences in age (p 0.273) or level of invasiveness between interviewed and noninterviewed cases. Histologically, interviewed cases were more likely to be of the serous subtype (57.4% for interviewed cases, 45.6% for noninterviewed cases) and less likely to be classified as "other epithelial" (10.5% for interviewed cases; 22% for noninterviewed cases). There was no statistically significant difference between interviewed and noninterviewed cases for the other histologic subtypes. Information on perineal talc use was missing in 7 cases.

Households with eligible women were identified through RDD methods, resulting in 2,327 controls identified and sent an introductory letter with a prompt list. Eighty of these women were later found not to meet the age requirement and 21 were ineligible due to residence outside the study area. Ten controls were excluded due to speaking a language other than English or Spanish. Two hundred fifty-two controls were excluded due to reporting bilateral oophorectomy, resulting in 1,964 controls that met the study criteria. Nineteen controls were too ill to participate and 358 were later found to have moved, changed phone numbers, or failed to answer after repeated efforts. Of the remaining 1,587 contacted controls, 465 refused to participate, resulting in 1,122 completed control interviews for a response fraction of 57% for total identified eligible controls. Information on perineal talc exposure was missing in 17 controls.

Invasive tumors constituted 71.1% of the case series and 28.9% of the tumors were of borderline malignancy. Among non-Hispanic white women, who constituted 74% of the cases, 25.8% were of borderline invasiveness. Overall, 57% of the case series were serous adenocarcinomas, divided 60% and 40% for invasive and borderline, respectively. Mucinous and endometrioid each comprised 14% of the EOC cases. There were slightly more mucinous borderline cases than invasive cases. Clear cell and other/unclassified histologies made up the remaining 5% and 11%, respectively.

The demographic characteristics of all cases and controls and cases and controls stratified by talc exposure are shown in Table I. Matching was successful and cases and controls were similar in age and ethnicity. Controls were less likely to have finished high school but more likely to have an education beyond high school. A somewhat larger proportion of the case series were single (12.5%) compared to the control series (10.0%). Control women were more likely to have been born outside of the United States (16.8%) than were cases (12.9%).

A total of 42.6% of EOC cases reported ever use of talcum powder in the perineal area while 37.1% of control women reported such a history. Case women using talc were slightly older at interview than controls. Women in the oldest age group used talc less than younger women, much more so for control women than case women. White non-Hispanic women were more likely to use talc than their Hispanic counterparts. Talc use was higher in both white non-Hispanic and Hispanic cases compared to controls but this pattern was not seen in the other ethnic category. Talc use was also associated with a higher education level. Talc use was higher in both cases and controls with birthplace in the United States.

TABLE I DESCRIPTIVE CHARACTERISTICS OF EOC CASES AND CONTROLS IN CALIFORNIA S CENTRAL VALLEY BY TALC EXPOSURE, 2000 2001

Characteristic	Cases		Controls	
	Total[1]	Talc exposure, *n* (%)	Total[1]	Talc exposure, *n* (%)
Number of subjects	249	106 (42.6)	1105	410 (37.1)
Mean age at interview	56.6	56.6	55.0	53.7
Age group (%)				
40	20	7 (35.0)	112	43 (38.4)
40 49	66	34 (51.5)	317	121 (38.2)
50 59	57	20 (35.1)	268	113 (42.2)
60 69	56	25 (44.6)	211	82 (38.9)
70	50	20 (40.0)	197	51 (25.9)
Ethnicity (%)				
White non-Hispanic	187	85 (45.5)	802	317 (39.5)
Hispanic	42	15 (35.7)	201	54 (26.9)
Other	20	6 (30.0)	102	39 (38.2)
Education (%)				
high school graduate	33	13 (39.4)	208	60 (28.8)
High school graduate	84	34 (40.5)	261	99 (37.9)
high school graduate	130	59 (45.4)	635	251 (39.5)
Marital status (%)				
Single	32	11 (34.4)	111	40 (36.0)
Married	133	59 (44.4)	670	246 (36.7)
Divorced/separated	40	17 (42.5)	175	77 (44.0)
Widowed	44	19 (43.2)	145	46 (31.7)
Birthplace (%)				
In United States	216	96 (44.4)	919	379 (41.2)
Outside United States	33	10 (30.3)	186	31 (16.7)

[1]Numbers may not add up to total cases and controls due to missing data.

TABLE II FREQUENCIES, MULTIVARIATE-ADJUSTED ODDS RATIOS AND 95% CONFIDENCE INTERVALS FOR PATTERNS OF TALC USE FOR EOC CASES AND CONTROLS, CENTRAL VALLEY OF CALIFORNIA, 2000 2001

Patterns of talc use	Cases (%) (*n* 256)[1]	Controls (%) (*n* 1,122)[1]	Multivariate-adjusted OR (95% CI)
Talc use			
Never	143 (57.4)	695 (62.9)	1.0
Ever	106 (42.6)	410 (37.1)	1.37 (1.02 1.85)
Frequency of use			
Never	143 (57.4)	695 (63.2)	1.0
Rarely to several times per month	34 (13.7)	138 (12.5)	1.34 (0.87 2.08)
1 3 times per week	31 (12.4)	145 (13.2)	1.16 (0.74 1.81)
4 7 times per week	41 (16.5)	122 (11.1)	1.74 (1.14 2.64)
			Trend *p* 0.015
Duration of use			
Never	143 (58.9)	695 (64.2)	1.0 (referent)
3 years	18 (7.4)	99 (9.2)	1.01 (0.58 1.76)
4 12 years	32 (13.2)	98 (9.1)	1.86 (1.16 2.98)
13 30 years	29 (11.9)	102 (9.4)	1.45 (0.90 2.32)
30 years	21 (8.6)	88 (8.1)	1.22 (0.72 2.08)
			Trend *p* 0.045
Cumulative use (frequency duration)			
Never	143 (58.9)	695 (64.4)	1.0 (referent)
First quartile (lowest exposure)	18 (7.4)	95 (8.8)	1.03 (0.59 1.80)
Second quartile	28 (11.5)	95 (8.8)	1.81 (1.10 2.97)
Third quartile	34 (14.0)	107 (9.9)	1.74 (1.11 2.73)
Fourth quartile (highest exposure)	20 (8.2)	88 (8.1)	1.06 (0.62 1.83)
			Trend *p* 0.051

Adjusted for age, race/ethnicity, duration of oral contraceptive use and breast feeding. [1]Numbers may not add up to total cases and controls due to missing data.

Ever use of talcum powder in the genital area was associated with a 37% elevation in risk of EOC, which was statistically significant (Table II). Increasing frequency of use was associated with increasing risk such that those women who reported use 4 7 times per week experienced a significant 74% elevation in EOC risk (p for trend 0.015). However, this was not a monotonic trend in that risk decreased between the second and third categories of use (from 1.34 to 1.16). Duration of use of talcum powder was associated with increased risk, although the pattern was also not clear-cut in that the point estimate peaked among those reporting 4 12 years of use and declined somewhat among those reporting longer duration of use (p for trend 0.045). Cumulative use also demonstrated an uneven association with risk of EOC in that the point estimates peaked in the second and third quartiles of intensity but declined in the highest quartile of use.

The multivariate adjusted odds ratios were elevated primarily among those with a serous or mucinous invasive tumor and were lower among women with other cell types or with borderline tumors (Table III).

Risk of EOC associated with use of talcum powder was higher and statistically significant in those who reported first using pow-

TABLE III FREQUENCIES, MULTIVARIATE-ADJUSTED ODDS RATIOS AND 95% CONFIDENCE INTERVALS FOR PERINEAL TALC USE AND EOC RISK BY INVASIVENESS AND HISTOLOGIC SUBTYPE, CENTRAL VALLEY OF CALIFORNIA, 2000 2001

Histologic subtype	Cases (%) (*n* 256)[1]	Controls (%) (*n* 1,122)[1]	Multivariate-adjusted OR (95% CI)
All invasive (*n* 182)[1]			
perineal talc use	98 (55.7)	696 (62.9)	1.0 (referent)
perineal talc use	78 (44.3)	410 (37.1)	1.51 (1.07 2.12)
Serous invasive (*n* 92)[1]			
perineal talc use	46 (52.3)	696 (62.9)	1.0 (referent)
perineal talc use	42 (47.7)	410 (37.1)	1.77 (1.12 2.81)
Mucinous invasive (*n* 16)			
perineal talc use	6 (37.5)	696 (62.9)	1.0 (referent)
perineal talc use	10 (62.5)	410 (37.1)	2.56 (0.89 7.39)
Endometrioid (*n* 35)			
perineal talc use	21 (60.0)	696 (62.9)	1.0 (referent)
perineal talc use	14 (40.0)	410 (37.1)	1.28 (0.62 2.62)
Clear cell (*n* 12)[1]			
perineal talc use	8 (72.7)	696 (62.9)	1.0 (referent)
perineal talc use	3 (27.3)	410 (37.1)	0.63 (0.15 2.64)
Other epithelial (*n* 27)[1]			
perineal talc use	17 (65.4)	696 (62.9)	1.0 (referent)
perineal talc use	9 (34.6)	410 (37.1)	1.06 (0.45 2.48)
All borderline (*n* 74)[1]			
perineal talc use	45 (61.6)	696 (62.9)	1.0 (referent)
perineal talc use	28 (38.4)	410 (37.1)	1.09 (0.65 1.83)
Serous borderline (*n* 55)[1]			
perineal talc use	32 (59.3)	696 (62.9)	1.0 (referent)
perineal talc use	22 (40.7)	410 (37.1)	1.28 (0.71 2.31)
Mucinous borderline (*n* 19)			
perineal talc use	13 (68.4)	696 (62.9)	1.0 (referent)
perineal talc use	6 (31.6)	410 (37.1)	0.76 (0.28 2.07)

Adjusted for age, race/ethnicity, duration of oral contraceptive use and breast feeding. [1]Numbers may not add up to total cases and controls due to missing data.

TABLE IV FREQUENCIES, MULTIVARIATE-ADJUSTED ODDS RATIOS AND 95% CONFIDENCE INTERVALS FOR PERINEAL TALC USE AND EOC RISK BY TIMING OF USE, CENTRAL VALLEY OF CALIFORNIA, 2000 2001

Timing of talc use	Cases (%) (*n* 256)[1]	Controls (%) (*n* 1,122)[1]	Multivariate-adjusted OR (95% CI)
Year of first use			
Never use	143 (59.1)	695 (65.6)	1.0 (referent)
Before/during 1975	52 (21.5)	206 (19.4)	1.22 (0.84 1.77)
After 1975	47 (19.4)	149 (15.0)	1.92 (1.27 2.91)
Age at first use			
Never use	143 (59.1)	695 (65.7)	1.0 (referent)
20 years	30 (12.4)	169 (16.0)	0.95 (0.61 1.48)
20 24 years	26 (10.7)	61 (5.8)	2.41 (1.43 4.09)
25 years	43 (17.8)	133 (12.6)	1.80 (1.19 2.73)
First use before or after first birth[2]			
Never use	113 (59.2)	631 (65.6)	1.0 (referent)
Use at or prior to first birth	36 (18.8)	229 (23.8)	0.98 (0.64 1.48)
Use after first birth	42 (22.0)	102 (10.6)	2.51 (1.63 3.87)
Years since last use			
Never use	143 (59.1)	695 (65.6)	1.0 (referent)
Current users	32 (13.2)	133 (12.5)	1.27 (0.81 1.98)
1 2 years	27 (11.2)	61 (5.8)	2.40 (1.43 4.05)
3 20 years	20 (8.3)	83 (7.8)	1.57 (0.90 2.73)
20 years	20 (8.3)	88 (8.3)	1.13 (0.66 1.94)

Adjusted for age, race/ethnicity, duration of oral contraceptive use and breast feeding. [1]Numbers may not add up to total cases and controls due to missing data. [2]Parous women only.

der after 1975 compared to those reporting use prior to that date (Table IV). Higher risk was found among those reporting first use at ages after age 20 compared to those who were younger at first use (Table IV). In addition, risk was elevated among those women who used talcum powder only after the birth of their first child, while no effect was seen among those whose first use occurred before their first child was born.

In an attempt to assess latency issues, we evaluated risk of EOC by categorizing participants by the numbers of years since last reported use of talcum powder (Table IV). The highest and significant risks were found among women who had stopped using talcum powder relatively recently (1 2 years prior to interview), while those who reported last using powders in the more distant past did not experience altered risk.

An evaluation of effect modification of the talcum powder-EOC relationship by gynecologic surgery, reproductive history, exogenous hormone use and BMI is presented in Table V. Women without a tubal ligation experienced higher talcum powder-associated risks than women with a tubal ligation and this result was statistically significant (OR 1.54; 95% CI 1.10 2.16). The interaction coefficient for the relationship between talc use and tubal ligation was not statistically significant. There was no mod-

TABLE V FREQUENCIES, MULTIVARIATE-ADJUSTED ODDS RATIOS AND 95% CONFIDENCE INTERVALS FOR TALC USE AND RISK OF EOC BY LEVELS OF MODIFIERS IN THE CENTRAL VALLEY OF CALIFORNIA, 2000 2001

	Cases (n 256)[1]	Controls (n 1,122)[1]	Multivariate-adjusted OR (95% CI)
Tubal ligation			
Never talc use	29 (56.9)	161 (54.9)	1.0 (referent)
Ever talc use	22 (43.1)	132 (45.1)	0.88 (0.46 1.68)
No tubal ligation			
Never talc use	113 (57.4)	531 (65.8)	1.0 (referent)
Ever talc use	84 (42.6)	276 (34.2)	1.54 (1.10 2.16)
Hysterectomy[2]			
Never talc use	27 (50.0)	117 (58.8)	1.0 (referent)
Ever talc use	27 (50.0)	82 (41.2)	1.79 (0.91 3.52)
No hysterectomy[3]			
Never talc use	116 (59.5)	576 (63.7)	1.0 (referent)
Ever talc use	79 (40.5)	328 (36.3)	1.33 (0.95 1.87)
Ever pregnant			
Never talc use	118 (55.9)	648 (63.0)	1.0 (referent)
Ever talc use	93 (44.1)	381 (37.0)	1.44 (1.05 1.97)
Never pregnant			
Never talc use	25 (65.8)	47 (62.7)	1.0 (referent)
Ever talc use	13 (34.2)	28 (37.3)	0.93 (0.37 2.34)
Ever parous[4]			
Never talc use	113 (57.4)	633 (62.7)	1.0 (referent)
Ever talc use	84 (42.6)	376 (37.3)	1.34 (0.97 1.85)
Nulliparous[4]			
Never talc use	5 (35.7)	15 (75.0)	1.0 (referent)
Ever talc use	9 (64.3)	5 (25.0)	4.91 (0.68 35.25)
Ever OC use			
Never talc use	72 (51.4)	422 (57.7)	1.0 (referent)
Ever talc use	68 (48.6)	309 (42.3)	1.26 (0.86 1.83)
Never OC use			
Never talc use	71 (65.1)	272 (72.9)	1.0 (referent)
Ever talc use	38 (34.9)	101 (27.1)	1.63 (1.0 2.64)
HRT[5]			
Never talc use	54 (52.4)	220 (59.9)	1.0 (referent)
Ever talc use	49 (47.6)	147 (40.1)	1.41 (0.89 2.24)
No HRT[6]			
Never talc use	89 (62.2)	472 (64.4)	1.0 (referent)
Ever talc use	54 (37.8)	261 (35.6)	1.30 (0.87 1.93)
BMI 25			
Never talc use	55 (63.2)	311 (66.5)	1.0 (referent)
Ever talc use	32 (36.8)	157 (33.5)	1.23 (0.74 2.04)
BMI 25			
Never talc use	85 (53.5)	358 (59.1)	1.0 (referent)
Ever talc use	74 (46.5)	248 (40.9)	1.36 (0.92 1.99)

Adjusted for age, race/ethnicity, duration of oral contraceptive use and breast feeding. [1]Numbers may not add up to total cases and controls due to missing data. [2]Includes women with 2 years since hysterectomy. [3]Includes women with 2 years since hysterectomy. [4]Gravida women only. [5]Includes women with one or more years of use. [6]Includes women with never use or 1 year of use.

ification within categories of prior hysterectomy, however. Higher risks were observed among those who were ever pregnant compared to those who were never pregnant. Talcum powder-associated risk was not different within the parous and nulliparous. Talcum powder-associated risk was higher (and significant) in women who never used oral contraceptives; however, the interaction coefficient was not statistically significant. Neither BMI or hormone replacement therapy (HRT) use appeared to modify the relationship of talc use and EOC risk.

DISCUSSION

The prevalence of talc use among controls in our study (37.1%) is similar to the average percentage of use among the control populations in a review of 14 studies (36.8% calculated from data presented in original study).[21] In the current analysis as in others,[11,21–24] a larger percentage of cases *versus* controls reported perineal exposure to talc. We found a slight trend of decreasing use with increasing age in control women but our findings were not as strong as those noted by Rosenblatt *et al*.[25] Other studies[21,24] have found increased use in both cases and controls over 50 years of age compared to their counterparts less than or equal to 50 years of age. In the present study, cases less than 50 years of age were more likely to have used talc *versus* women 50 years or older (47.7% and 39.9%, respectively). Different findings in talc use patterns between the present study and previous studies may be explained by differences in study locations, study time periods and age categories. Frequency of use in the current study was similar for both the younger and older groups in controls (38.2% and 36.4%, respectively).

Talc use was higher in white non-Hispanics compared to Hispanics in this study. However, the pattern of increased use in EOC cases for both groups contributed to the overall increased risk of EOC among talc users. Differential talc use by various ethnic groups and its relation to EOC risk has not, to our knowledge, been evaluated previously.

As in other studies,[21,25] we found that talc use increased with education level, although one earlier study reported the opposite finding.[24] Other studies have compared talc use in ever married to never married women and found either similar use in both groups for cases and controls[21] or increased frequency of use in ever married women.[24] In the current analysis, talc exposure was 43.8% for ever married cases *versus* 34.4% for never married cases. However, fre-

quency of use was similar between ever married and never married controls (37.2% and 36.0%, respectively). There was much greater use of talc among those born in the United States *versus* those born outside it.

The odds ratio comparing ever use to never use in this study (OR 1.37; CI 1.02 1.85) is similar to the results of a recent metaanalysis that pooled 16 studies (summary RR 1.33; CI 1.16 1.45).[4] When stratified by hospital- *versus* population-based studies, the population studies had a summary relative risk of 1.38 (1.25 1.52).

Cornstarch use and ovarian cancer has been evaluated in a small number of case-control studies[11,21,22,26] and have been reviewed with the conclusion that no relationship exists between cornstarch and EOC, although the number of study participants using cornstarch *versus* talc was small.[13] Our study was not able to differentiate between use of perineal powders containing talc and those containing cornstarch, which may have driven the odds ratio toward the null. Type of application, including direct application on the perineum, or indirect exposure from dusting sanitary napkins, underwear and diaphragms (storage) was also not assessed.

As in other studies,[4] the present study did not find a clear dose response based on duration of use or cumulative use. Limiting the analysis of dose response to women who reported ever use of talc did not affect the results (data not shown). The lack of dose response between talc use and EOC may be explained by the inability to quantify the actual amount of talc used per application and timing of the application.[21] Cramer *et al.*[21] propose that application during ovulation may pose more risk due to the possibility of talc entrapment in inclusion cysts. Harlow *et al.*[24] found little change in odds ratios after excluding use after tubal ligation or hysterectomy in their estimate of total lifetime perineal talc applications. However, when they excluded nonovulatory periods of exposure in their calculation, there was significant increase in risk. We were unable to exclude nonovulatory periods and talcum powder use after gynecologic surgery in our cumulative use calculations.

Our analysis found that talc use and EOC risk varied by histologic subtype, as have others who found that exposure to talc is a significant risk for invasive tumors[22] and specifically for serous invasive tumors.[21] Cook *et al.*[11] also found an increased risk of serous tumors (including both invasive and borderline) in talc users *versus* nonusers. Gertig *et al.*[27] have suggested that there are pathologic similarities between serous adenocarcinomas and mesothelioma that may explain findings of increase risk for serous invasive tumors in talc users. Harlow *et al.*[24] reported a significant increase in risk of either endometrioid or borderline tumors with talc use and suggest that variation in risk among histologic subtypes may be due to chance or a foreign-body effect unique to specific subtypes.

In a study of the mineral and chemical characterization of consumer talcum powder formulated prior to June 1973, almost half of the samples tested contained 1 of the 3 asbestos group minerals.[1] In 1976, talcum powder manufacturers instituted voluntary guidelines to prevent asbestos contamination in talc products,[24] but we did not find an increase in EOC risk with talc use on or before 1975; rather, we found that risk of EOC increased with use after 1975, which may be related to the recency of use. Harlow *et al.*[24] observed ovarian cancer risk was increased in women using talc products before 1960, although Chang and Risch[22] found no relationship between risk and use either before or after 1970.

In the current analysis, a statistically significant increase in EOC risk occurred with first use after age 20 compared to first use at younger ages. Controlling for recency of use did not change this finding. Other studies have reported either no trend with age at first use[21] or increased risk of EOC with first use at younger than age 20 and older than age 25.[24] Disagreement in findings between studies may be due to differences in age distributions and talc use patterns among study participants. Although we cannot directly assess risk during ovulatory *versus* nonovulatory periods, our findings of increased risk in adult women support the hypotheses of increased EOC risk with talc exposure during ovulatory periods and in parous reproductive tracts.

Cramer *et al.*[21] found that EOC risk was increased in parous women with talc use occurring before first birth, suggesting that prepregnancy ovarian tissue may be more vulnerable to talc damage because it has not undergone stromal differentiation (decidual reaction that occurs during pregnancy). However, their reference group was all parous women. In this analysis, we stratified parous women by never use, use before first birth and use after first birth. We found increased risk after first pregnancy. Anatomical changes in the genital tract after pregnancy may increase the possibility of talc migration to the ovaries.[28] Harlow *et al.*[24] suggest that pregnancy may increase risk due to its effect of increasing the size of the cervical opening into the uterus. In the current study, perineal talc use had no apparent impact on EOC risk among those women who had never been pregnant and parity was difficult to evaluate because of the small number of nulliparous women. In 2 prospective studies of talc use and EOC risk, there was no significant difference in parity between users and nonusers of talc.[25,27]

Harlow *et al.*[24] reported a significant increase in EOC risk if perineal talc use occurred in the last 6 months. We also found that recent users were at increased risk (even when we controlled for duration of use). It is noteworthy that a significant latency effect is well documented for asbestos exposure and development of both pleural and peritoneal mesotheliomas[29] while there appears to be no latency with talcum powder. The asbestos association has been reported from an occupational cohort mortality study where exposure is indirect and not the result of direct application, as is the case for talcum powder.[30] This may explain the differences between observed patterns of latency for asbestos and talcum powder. Additionally, risk of EOC with talc use may not be due to talc s chemical similarity to asbestos but rather due to the ovary s unique function, resulting in vulnerability to carcinogenesis from particulates such as talc.[8] In a study of gynecologic surgery and EOC, Green *et al.*[31] found that women reporting fallopian tubal occlusion, through tubal ligation or hysterectomy, were at decreased risk for developing EOC. They concluded that surgical tubal occlusion decreased EOC risk by preventing contaminants from reaching the ovary. Ness and Cottreau[32] proposed that the inflammatory response of the ovarian epithelium to various irritants may result in ovarian mutagenesis, tumor growth and tumor invasiveness. Cramer *et al.*[21] reported no association between EOC risk and talc use in women with a tubal ligation; however, risk remained nonsignificantly elevated in women with a hysterectomy. A recent prospective study[27] found that EOC risk in talc users was not modified by either tubal ligation or hysterectomy. The analysis was not able to determine the timing of talc use (before or after surgery). In a hospital-based case-control study, Wong *et al.*[33] found that risk of EOC with talc use was increased in women without gynecologic surgery and decreased in women with a history of tubal ligation or hysterectomy but neither finding was significant. They also were unable to delineate use before or after gynecologic surgery. Tubal ligation may limit a woman s exposure to contaminants more than hysterectomy since it is usually performed earlier in a woman s reproductive history, while she is still ovulating.[34]

Oral contraceptives (OCs) act by suppression of ovulation and the fact that elevated risks were found in those talcum powder users that never used OCs in this study suggests that uninterrupted ovulation with associated formation of inclusion cysts may enhance the impact that talcum powder may have on ovarian carcinogenesis. Unlike our study, however, Cramer *et al.*[21] and Harlow *et al.*[24] reported that OCs had no effect on talc use and EOC risk. A prospective study of talc use and ovarian cancer also found that the prevalence of OC use was similar in both users and nonusers of talc. However, these studies also reported lower percentages of OC use among both cases and controls (talc users and nonusers) than was found in the present study.

Our analysis found that BMI did not modify the risk associated with talc use and EOC in agreement with Cramer *et al.*[21] Talc use

was greater in women with a high *versus* low BMI for both cases and controls but the difference was not significant. Rosenblatt *et al*.[25] in a prospective study found that women in the highest BMI quartile were more likely to use perineal talc. They concluded that since some studies have found an increased risk for ovarian cancer in obese women, BMI may be a confounder of talc use and ovarian cancer risk. Harlow *et al*.,[24] however, reported no differential use of talc between leaner and heavier controls.

There are several limitations to this study that may limit interpretation of the findings. The sample size was relatively small and the response fraction lower than ideal. However, we have observed the same or similar relationships in our study between several risk factors such as OC use and parity, as has been observed in several earlier studies. Recall bias has also been implicated as a limitation in studies of talc and ovarian cancer.[35] However, findings in a prospective study, the Nurses Health Study, in which exposure data were collected prior to diagnosis and hence free of recall bias, were similar to the present study finding for talc use and serous invasive ovarian cancer.[27] It has also been suggested that use of talc is habitual *versus* memorable and not likely to be subject to recall bias.[35] Huncharek *et al*.[4] suggested that the positive relationship between talc use and EOC risk found in a review of epidemiologic studies may also be explained by a treatment effect in prevalent cases. The present study used incident cases exclusively. The present analysis was also limited due to our inability to exclude use during nonovulatory periods and posttubal ligation or hysterectomy, nor were we able to differentiate between various formulations.

Research has provided little biologic or experimental evidence to support a relationship between talcum powder use and ovarian cancer risk. However, given the suggestive though uncertain role of talcum powder and EOC found in epidemiologic studies, including the present study, users should exercise prudence in reducing or eliminating use. In this instance, the precautionary principle should be invoked, especially given that this is a serious form of cancer, usually associated with a poor prognosis, with no current effective screening tool, steady incidence rates during the last quarter century and no prospect for successful therapy. Unlike other forms of environmental exposures, talcum powder use is easily avoidable.

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Exhibit 58

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Markers of inflammation and risk of ovarian cancer in Los Angeles County

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Factors that increase inflammation have been suggested to influence the development of ovarian cancer, but these factors have not been well studied. To further investigate this question, we studied the role of talc use, history of endometrioisis and use of non-steroidal anti-inflammatory drugs (NSAIDs) and risk of ovarian cancer in a population-based case-control study in Los Angeles County involving 609 women with newly diagnosed epithelial ovarian cancer and 688 population-based control women. Risk of ovarian cancer increased significantly with increasing frequency and duration of talc use; compared to never users risk was highest among long-duration (20+ years), frequent (at least daily) talc users (adjusted relative risk (RR) = 2.08, 95% confidence interval (CI) = 1.34–3.23). A history of physician-diagnosed endometriosis was statistically significantly associated with risk (RR = 1.66, 95% CI = 1.01–2.75). Women who were talc users and had a history of endometriosis showed a 3-fold increased risk (RR = 3.12, 95% CI = 1.36–7.22). Contrary to the hypothesis that risk of ovarian cancer may be reduced by use of NSAIDs; risk increased with increasing frequency (per 7 times/week, RR = 1.27, 95% CI = 1.14–1.43) and years of NSAID use (per 5 years of use, RR = 1.25, 95% CI = 1.10–1.42); this was consistent across types of NSAIDs. We conclude that risk of ovarian cancer is significantly associated with talc use and with a history of endometriosis, as has been found in previous studies. The NSAID finding was unexpected and suggests that factors associated with inflammation are associated with ovarian cancer risk. This result needs confirmation with careful attention to the reasons for NSAID use.

Key words: talc; endometriosis; non-steroidal anti-inflammatory drugs; ovarian cancer

In 1999, Ness and Cottreau proposed that chronic inflammation may lead to the development of epithelial ovarian cancer.[1] They hypothesized that factors including talc exposure, endometriosis and pelvic inflammatory disease (PID) may increase risk by a common pathway, increasing local inflammation of the "ovarian epithelium." They also suggested that studying the effect of non-steroidal anti-inflammatory drugs (NSAIDs) may offer additional opportunities to evaluate the inflammation hypothesis. In a 2008 paper, Merritt *et al.*[2] studied the role of inflammation, based on histories of talc use, PID, endometriosis and use of NSAIDs in the same study. They concluded that chronic inflammation is unlikely to play an important role because risk of ovarian cancer was modestly increased in association with talc use and history of endometriosis and was unrelated to use of NSAIDs but they restricted attention to medication use in the 5 years prior to diagnosis of ovarian cancer, rather than long-term use.[2] No support for the use of NSAIDs was found in a recent study conducted in Seattle, Washington which collected information on lifetime medication use. These investigators found increased risk of ovarian cancer in association with use of acetaminophen, aspirin and other NSAIDs, particularly among long (10+ years) term users.[3] We have conducted a population-based case-control study of ovarian cancer in Los Angeles County to further investigate the role of inflammation in the risk of ovarian cancer. We focused our attention on risk in relation to lifetime use of talc, NSAIDs and history of various gynecological conditions. We are particularly interested in risk patterns associated with long dnration of NSAID use. We report our results herein.

Material and methods

Study design

This was a population-based case-control study of ovarian cancer. Eligible patients were English speaking residents of Los Angeles County between the ages of 18 and 74 inclusive who had histologically confirmed invasive or borderline (low malignant potential; LMP) ovarian cancers that were first diagnosed from 1998 to 2002. The cases were identified by the Cancer Surveillance Program (CSP), part of the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) Program, covering all residents of Los Angeles County.

A total of 1,097 patients meeting the pathological case definition were identified by the CSP. Of these, 136 patients had died or were too ill to be interviewed by the time we contacted them, 109 patients had moved away from Los Angeles County and could not be interviewed in person or they could not be located and 151 patients declined to be interviewed. Interviews were conducted with 701 ovarian cancer patients of whom 15 were later identified who did not have ovarian cancer and they were excluded from all analyses. Of the 686 ovarian cancer patients interviewed, 77 had a previous cancer (excluding non-melanoma skin cancer) before their diagnosis of ovarian cancer and were excluded from this report because their previous cancer diagnosis and/or treatment may have influenced use of NSAIDs and other risk factors. This left 609 ovarian cancer cases for the present analysis, 81% were invasive tumors [22% localized stage (Stage 1 or 2), 59% advanced stage (Stage 3 or greater) and 19% were LMP tumors. The cell type distribution is as follows: 58% serous, 14% clear cell/endometrioid, 12% mucinous and 16% other category.

Controls were identified through a well-established neighborhood recruitment algorithm, which we have used successfully in previous studies of breast, endometrial and other cancers to investigate the role of hormonal and non-hormonal medications and other factors.[4] For this study, controls were women with at least one intact ovary, with no history of cancer, except possibly non-melanoma skin cancer, and individually matched with patients on race/ethnicity (non-Hispanic White, African-American, Hispanic, Asians) and date of birth (+/−5 years). Neighborhood controls were sought by one of our staff who physically canvassed the neighborhood of the case using a systematic algorithm based on the address of the case. If the first eligible matched control declined to participate, the second eligible matched control in the sequence was asked, and so on. Letters were left when no one was at home, and follow-up by mail, telephone and further visits to the neighborhood continued until either an eligible control agreed to be interviewed or 150 housing units had been screened. When we failed to identify an exact race/ethnicity matched control, we

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accepted a control subject who was matched on age. A total of 688 control women were successfully interviewed by the closing date of the study. The first eligible match was interviewed for 76% of the patients, and the second match for another 17% and the third or later match for 6% of the patients. On average, we contacted a median number of 19 housing units to interview a matched control subjects for those neighborhoods with no refusal, a median of 36 housing units for those neighborhoods with 1 refusal and 58 housing units when there were 2 or more refusals.

Study participants were interviewed using a comprehensive questionnaire that covered medical, gynecological, reproductive and lifestyle history. All but 15 participants were interviewed in-person; cases and their matched controls were interviewed by the same person in almost all instances. A reference date was defined as 2 years before the date of diagnosis of the case. This same reference date was used for each case's matched control subject. Calendars were used to chart major life events and reproductive and contraceptive histories. Specifically, participants were asked if they were ever told by a physician that they had certain gynecological conditions including PID, gonorrhea, endometriosis, ovarian cysts, or uterine fibroids before the reference date. If the response was yes to any of the conditions, participants were then asked the age at which they were first diagnosed with the condition and if they had ever been treated for the condition. To determine the use of talcum powder, participants were asked if they ever used talc at least once per month for 6 months or more. If the response was positive, we then asked whether they had ever used talc in nonperineal areas (feet, arms, chest or back), perineal areas, or on underwear or sanitary pads/diaphragm. Questions on talc use included age at first use, frequency of use (times per month) and years of talc use. Few of the talc users (13 cases, 11 controls) had a tubal ligation or hysterectomy before they started using talc; the numbers were too sparse to determine for certain the effect of talc use in this group and these 24 users were included with the nonusers in subsequent analyses on frequency and duration of talc use. Results were unchanged when we excluded these 24 users from the analysis (data not shown).

We asked the participants whether they took prescription or nonprescription NSAIDs for various conditions including back trouble, arthritis, headaches, migraine headaches, dental problems, sinus trouble, colds or sore throats, menstrual pain or cramps or any other reason. They were also asked if they took any of these medications for prevention reasons, such as for prevention of heart attack. We explicitly asked about usage patterns of 10 common over-the-counter NSAIDs (regular aspirin, buffered aspirin, Anacin, APC, Tylenol, Excedrin, Advil, Nuprin, Coricidin, Dristan), 12 prescription brand-name NSAIDs (Clinoril, Motrin, Anaprox, Feldene, Empirin with codeine, Tylenol with codeine, Darvocet, Indocin, Fiorinal, Percocet-5, Percodan, Naprosyn) and two COX-2 inhibitors (Celebrex, Vioxx). We also asked the participants if they had used any NSAIDs that were not on our list and recorded the drug name and details of use. Respondents were also asked about use of 4 common diuretics; these medications are not hypothesized to be related to ovarian cancer risk, but they were included as a check of differential recall between cases and controls. Taking a specific medication 2 or more times a week for 1 month or longer was categorized as "use"; otherwise participants were considered "non-users." Participants were asked about the ages at first and last use, duration of use, usual frequency of use and the primary reason for such use. All of the medications data were categorized into the following groups based on their components: aspirin, acetaminophen, other NSAIDs, COX-2 inhibitors and diuretics.

Total duration and frequency of the main classes of medication (aspirin, acetaminophen, other NSAIDs) were calculated by summing all use of the same class of medication for each person (there were few users of COX-2 inhibitors, thus results are not shown). We also created a combined variable representing use of all NSAIDs. Duration of use was categorized as no use, less than 5 years, 5–10 years and >10 years of use of the specific type of medication (years of use of different medications may be overlapping). The no use category included never users, occasional users and those who only started to use a particular medication within the interval beginning 2 years before date of diagnosis for case patients and the same reference period for controls to avoid including medication use because of early symptoms in cancer patients. We also repeated the analyses excluding first use of medication within 5 years of diagnosis. In addition, we evaluated effect modification of the NSAIDs-ovarian cancer association by race/ethnicity, education, menopausal status, tumor stage, history of endometriosis, talc use and frequency of Pap smears in the 10 years before reference date.

TABLE I – DEMOGRAPHIC AND OTHER CHARACTERISTICS OF OVARIAN CANCER PATIENTS AND CONTROLS

	Cases $N = 609$	Controls $N = 688$	RR	95% CI[1]
Race/ethnicity				
Non-Hispanic White	381	503		
Black	41	44		
Hispanic	136	103		
Asian	51	38		
Age				
≤34	40	36		
35–44	92	138		
45–54	162	227		
55–64	149	162		
65+	166	125		
Education				
≤high school	92	50		
Some college	109	81		
College graduate	223	242		
Graduate	185	315		
Family history of ovarian cancer				
No	581	672	1.00	
Yes	26	16	1.76	0.89–3.47
p-value			0.10	
Number of livebirth				
0	156	149	1.00	
1	98	110	0.76	0.52–1.12
2	157	202	0.61	0.43–0.86
3	109	118	0.61	0.41–0.90
4+	89	109	0.34	0.22–0.53
p trend			<0.0001	
Oral contraceptives				
0 yr	241	189	1.00	
>0 to <5 yr	259	261	0.98	0.73–1.32
≥5 to <10 yrs	57	112	0.54	0.36–0.82
≥10 yrs	52	126	0.40	0.26–0.61
p trend			<0.0001	
Tubal ligation				
No	531	553	1.00	
Yes	78	135	0.66	0.47–0.93
p value			0.017	

[1]Adjusted for race/ethnicity, age, education, tubal ligation, family history of ovarian cancer, menopausal status, use of oral contraceptives, and parity.

The study was approved by the Institutional Review Board of the Keck School of Medicine at the University of Southern California. Informed consent was obtained from each case and control before her interview.

Statistical methods

We calculated odds ratios as estimates of relative risk (RR), their corresponding 95% confidence intervals (CIs) and statistical significance (p) values. We used conditional stratified logistic regression analysis, with stratification sets defined jointly by age (<35, 35–44, 45–54, 55–64, ≥65) and race/ethnicity (non-Hispanic White, African-American, Hispanic, Asians). All regression models also included as categorical covariates education level (high school or less, some college, college graduate, >college),

TABLE II – MULTIVARIABLE RRS (95% CIS) FOR TALC USE AND RISK OF OVARIAN CANCER

	Cases	Controls	RR	95% CI[1]
Talc use				
No[2]	363	469	1.00	
Yes	242	219	1.48	1.15–1.91
Yes, non-perineal area[3]	112	103	1.43	1.03–1.98
Yes, perineal area	130	116	1.53	1.13–2.09
Frequency and duration of talc use				
No	363	469	1.00	
1 ≤20 yrs and ≤10 times/month	35	31	1.36	0.79–2.32
1 ≤20 yrs and >10 to ≤30 times/month	23	30	1.16	0.63–2.12
1 ≤20 yrs and >30 times/month	21	21	1.23	0.63–2.41
>20 yrs and ≤10 times/month	45	49	1.27	0.80–2.01
>20 yrs and >10 to ≤30 times/month	51	43	1.57	0.99–2.50
>20 yrs and >30 times/month	67	45	2.08	1.34–3.23
p (6 df)				$p = 0.032$
Total times of talc use				
No	363	469	1.00	
≤5200	49	52	1.20	0.77–1.88
>5200 to ≤15600	46	47	1.38	0.87–2.20
>15,600 to ≤52000	63	61	1.34	0.89–2.02
>52000	84	59	1.99	1.34–2.96
p (1 df)				$p = 0.0004$
Total times of talc use				
No	363	469	1.00	
Before 1975				
≤5200	24	35	0.84	0.47–1.51
>5200 to ≤15600	29	29	1.41	0.79–2.53
>15,600 to ≤52000	49	45	1.45	0.91–2.31
>52000	82	58	1.93	1.29–2.88
After 1975				
≤5200	25	17	1.95	0.98–3.89
>5200 to ≤15600	17	18	1.17	0.56–2.48
>15,600	16	17	0.98	0.45–2.13

[1]Adjusted for race/ethnicity, age, education, tubal ligation, family history of breast/ovarian cancer, menopausal status, use of oral contraceptives and parity.–[2]Subjects (13 Cases, 11 Controls) reported tubal ligation and/or hysterectomy before started talc use and were included with the never users.–[3]Included arms and extremities.

age at menarche (<=11, 12, 13, 14+), parity (0, 1, 2, 3, 4+ births), use of oral contraceptives (none, >0 to <5, 5 to <10, 10+ years), family history of breast/ovarian cancer (no/yes), menopausal status (premenopausal, natural or surgical menopause) and tubal ligation (no/yes). Results obtained using stratified conditional logistic regression methods were consistent with those obtained in matched analyses that preserved the original case-control matching, and we show the results from the stratified analyses. All statistical significance p values quoted are two-sided and are standard chi-squared tests based on differences in log-likelihoods.

Results

The race/ethnicity, age and education of the ovarian cancer cases and controls are shown in Table I. Risk of ovarian cancer increased in association with family history of ovarian cancer (RR = 1.76, 95% CI = 0.89–3.47) and decreased significantly with increasing number of births (RR per birth = 0.79, 95% CI = 0.72–0.88), with increasing duration of oral contraceptive use (RR per 5 years of use = 0.73, 95% CI = 0.64–0.83) and with a history of tubal ligation (RR = 0.66, 95% CI = 0.47–0.93).

Table II shows risk associations with talc use. Ever use of talc was associated with a statistically significant increased risk (RR = 1.48, 95% CI = 1.15–1.91). This included talc that was applied to the perineal area (RR = 1.53, 95% CI = 1.13–2.09) and to the nonperineal area only (RR = 1.43, 95% CI = 1.03–1.98). Elevated risks were found among those who used talc on sanitary napkins (RR = 1.61, 95% CI = 0.93–2.78), underwear (RR = 1.71, 95% CI = 0.99–2.97) and on diaphragm/cervical caps (RR = 1.14, 95% CI = 0.46–2.87). When we examined risk patterns by frequency and duration of talc use, the effect of frequency of use was relatively modest among users of less than 20 years but there was a clear trend of increasing risk with increasing frequency of use among longer duration (>20 years) users. Compared with never users, risk was highest in long-term (>20 years), daily (>30 times/month) talc users (RR = 2.08, 95% CI = 1.34–3.23). Risk increased significantly with lifetime total times of talc use, but the association was limited to those who started talc use before 1975 (p_{trend} <0.001). The association between talc use and risk of ovarian cancer was strongest for serous ovarian cancer, the RR associated with any use was 1.70 (95% CI = 1.27–2.28). The risk associations for talc use and other histologic cell types overlapped with the finding for serous ovarian cancer (RRs were 0.99 for mucinous, 1.19 for clear/endometrioid and 1.46 for other cell types). Elevated risks in relation to talc use were found for those with invasive cancers (RR = 1.31, 95% CI = 0.85–2.01 for localized stage; RR = 1.66, 95% CI = 1.22–2.26 for advanced stage) and LMP tumors (RR = 1.32, 95% CI = 0.88–2.22).

Women with a history of physician-diagnosed endometriosis experienced a nearly 2-fold increased risk of ovarian cancer (RR = 1.95, 95% CI = 1.20–3.17). The risk associated with endometriosis remained statistically significant after adjustment for other gynecological conditions including PID, gonorrhea, ovarian cysts and uterine fibroids (adjusted RR = 1.66, 95% CI = 1.01–2.75) (Table III). Small (4–18%) increased risks were also associated with a history of the other gynecological conditions as mentioned earlier but none of these findings were statistically significant (data not shown). The risk of ovarian cancer increased significantly (RR = 2.58) for more recent diagnoses of endometriosis (2–10 years prior to cancer diagnosis) and was less strong (RR = 1.58) for women with diagnosis more than 10 years previously. The endometriosis-risk association was stronger for invasive cancers (RR = 1.80, 95% CI = 0.85–3.80 for localized stage, RR =

TABLE III – MULTIVARIABLE RRS (95% CIS) FOR PREVIOUS OVARIAN DISEASE AND RISK OF OVARIAN CANCER

		Cases	Controls	Adjusted RR[1]	Adjusted RR[2]
Pelvic inflammatory disease					
No		579	657	1.00	1.00
Yes		25	22	1.48 (0.78–2.82)	1.15 (0.60–2.21)
Gonorrhea					
No		553	619	1.00	1.00
Yes		51	60	1.19 (0.77–1.84)	1.04 (0.67–1.62)
Endometriosis					
No		553	642	1.00	1.00
Yes		51	37	1.95 (1.20–3.17)	1.66 (1.01–2.75)
Years since first diagnosed					
2–10		15	8	2.66 (1.06–6.64)	2.58 (1.03–6.48)
11+		36	29	1.56 (0.90–2.70)	1.58 (0.91–2.76)
Talc	Endometriosis				
No	No	332	435	1.00	1.00
No	Yes	29	28	1.68 (0.93–3.04)	1.67 (0.92–3.01)
Yes	No	221	207	1.50 (1.15–1.94)	1.49 (1.15–1.94)
Yes	Yes	22	9	3.17 (1.38–7.29)	3.12 (1.36–7.22)
p (3df)					0.001

[1]Adjusted for race/ethnicity, age, education, tnbal ligation, family history of breast/ovarian cancer, menopansal status, use of oral contraceptives and parity.–[2]Adjusted for other conditions including pelvic inflammatory diseases, gonorrhea, endometriosis, ovarian cyst and fibroids.

1.87, 95% CI = 1.04–3.35 for advanced stage) than for LMP tumors (RR = 1.28, 95% CI = 0.56–2.95). Although the point risk estimate was slightly higher for clear/endometrioid cancers (RR = 1.97), the risk associations for the other cell types were all aronnd 1.70. Compared with women who did not have endometriosis and were nontalc users, risk increased 3-fold (RR = 3.12, 95% CI = 1.36–7.22) in women who had endometriosis and were talc users whereas about 50% increased risk was observed in women who had either exposure.

Risk of ovarian cancer increased significantly with increasing duration and frequency of use of all NSAIDs (*i.e.*, aspirin, acetaminophen, other NSAIDs). The risk per 5 years of NSAID use was 1.25 (95% CI = 1.10–1.42) and the risk per 7 times of NSAID nse per week was 1.27 (95% CI = 1.14–1.43). The effect of total pill use was essentially identical to the effect of frequency of use. This pattern of risk elevation was fonnd for aspirin, acetaminophen and other NSAIDs although the results were statistically significant only for other NSAIDs (Table IV). Risks patterns remained essentially unchanged when we adjusted for indication of use (*i.e.*, headaches, back pain, menstrual pain and others) or history of endometriosis and other gynecological conditions. Risk associations were only slightly reduced when we restricted our analyses to inedication use at least 5 years before diagnosis; the RR per 5 years of all NSAID use was 1.20 (95% CI = 1.06–1.43) and the risk per 7 times of NSAID use per week was 1.23 (95% CI = 1.09–1.38). In contrast, risk of ovarian cancer was not significantly related to duration or frequency of use of diuretics (RRs were 1.00, 1.39, 0.89, 0.65, respectively for no, 1–5, >5–10, >10 years of use, $p_{trend} = 0.50$).

Table V presents stratified resnlts, when we performed a series of analyses to evaluate whether the findings with NSAID use were consistent across levels of various subgroups of interest including race/ethnicity, education, menopausal status, tumor stage, endometriosis, talc use, use of oral contraceptives, parity and frequency of Pap smears in recent 10 years as a marker of access to care. Elevated risks in relation to NSAID use were found in all the subgroup analyses; findings were similar by race/ethnicity, menopausal status, talc use, oral contraceptive use, parity and history of Pap smear. There were some differences in risk estimates by education, tumor stage, history of endometriosis but they were not statistically significantly different. We considered these differences by tumor stage, history of endometriosis and education in our interpretation of these results.

Discussion

The main objective of this population-based case-control study was to comprehensively investigate the role of inflammation in risk of ovarian cancer by studying factors that have been hypothesized to increase inflammation (*e.g.*, talc, endometriosis) or to reduce inflammation (NSAIDs) simultaneously in the same population. Our findings on talc and endometriosis are consistent with previous findings and are compatible with the hypothesis that these factors increase the risk of ovarian cancer and that inflammation may be a common pathway.[1,2,5] However, contrary to the study hypothesis that NSAIDs may have chemopreventive effects by decreasing inflammation,[6] we found that risk of ovarian cancer increased significantly with increasing frequency and dnration of NSAIDs use.

Our results on NSAID and risk are similar to the recent results reported in the population-based case-control study conducted in Seattle, Washington.[3] In both stndies, women were asked to recall prescription and nonprescription medicatious taken over their lifetime for various conditions. In the Seattle study, risk of ovarian cancer increased significantly in association with 10+ years of use of acetaminophen (RR = 1.8, 95% CI = 1.3–2.6), aspirin (RR = 1.6, 95% CI = 1.1–2.2) and other NSAIDs (RR = 1.3, 95% CI = 1.0–1.7).[3] Mechanisms whereby use of NSAID may increase risk of ovarian cancer may be related, in part, to the underlying conditions associated with medication nse.

However, our results and those from the Seattle study differed from most previous studies on this topic. As Cramer *et al.* reported risk reduction of ovarian cancer with ever use of aspirin, and acetaminophen, but not with use of ibuprofen,[7] 7 (3 case-control, 4 cohort) of 13 (7 case-control, 6 cohort) studies have found no significant relation with use of NSAID. The case-control studies showing null findings were conducted in Italy,[8] the UK[9] and Australia,[2] and they investigated risk associations with use of aspirin,[8] acetaminophen and other NSAID,[9] and aspirin and other NSAIDs,[2] respectively. There was also no relationship between acetaminophen use and risk in the Cancer Prevention II Mortality Study[10] or between risk and use of low-dose aspirin[11] and other NSAIDs[12] in a Danish prescription database study. In the Breast Cancer Detection Demonstration Project Follow-up Study (BCDDP), risk was not significantly related to use of aspirin, acetaminophen and other NSAIDs but risk was increased with 5+ years of other NSAID use (RR = 2.0, 95% CI = 0.95–4.2).[13] Six other studies (4 case-control, 2 cohort) are supportive of an inverse

TABLE IV – MULTIVARIABLE RRS[1] (95% CIS) FOR USE OF ALL NSAIDs (ASPIRIN, ACETAMINOPHEN, OTHER NSAIDs) AND RISK OF OVARIAN CANCER

	Excluded medication use the 2 years before reference date		
	Cases	Controls	RR (95% CI)
All NSAIDs			
Years of use			
Never[2]	355	486	1.00
1 to 5 yr	117	99	1.71 (1.23–2.39)
>5 to ≤10 yr	37	33	1.59 (0.93–2.72)
>10 yr	79	57	1.81 (1.21–2.71)
p trend			<0.001
No. of pills per week			
Never[2]	355	486	1.00
1 to ≤7/wk	82	66	1.62 (1.11–2.39)
>7 to ≤14/wk	41	49	1.09 (0.67–1.78)
>14/wk	110	74	2.24 (1.56–3.21)
p trend			<0.001
Total no. of pills			
Never	355	486	1.00
1 to ≤1096	73	63	1.60 (1.08–2.38)
>1096 to 6428	73	66	1.43 (0.96–2.13)
>6428	87	60	2.22 (1.49–3.31)
p trend			<0.001
Years of use by type[3]			
Aspirin			
Never[2]	492	597	1.00
1 to 5 yr	46	25	2.13 (1.21–3.77)
>5 to ≤10 yr	13	18	0.70 (0.31–1.58)
>10 yr	31	28	1.15 (0.62–2.13)
p trend			0.43
Acetaminophen			
Never[2]	491	590	1.00
1 to 5 yr	47	53	0.87 (0.53–1.41)
>5 yr	44	25	1.71 (0.94–3.09)
p trend			0.12
Other NSAIDs			
Never[2]	450	575	1.00
1 to 5 yr	87	61	1.76 (1.18–2.63)
>5 to ≤10 yr	17	19	1.18 (0.55–2.53)
>10 yr	28	13	2.18 (1.03–4.63)
p trend			0.008
Frequency of use by type[3]			
Aspirin			
Never[2]	492	597	1.00
1 to ≤7/wk	61	48	1.49 (0.94–2.35)
>7	29	23	1.18 (0.61–2.29)
p trend			0.21
Acetaminophen			
Never[2]	491	590	1.00
1 to ≤7/wk	48	45	1.04 (0.63–1.71)
>7 /wk	43	33	1.36 (0.78–2.36)
p trend			0.33
Other NSAIDs			
Never[2]	450	575	1.00
1 to ≤7/wk	52	38	1.56 (0.95–2.56)
>7 to ≤14/wk	29	25	1.27 (0.68–2.40)
>14/wk	51	30	2.22 (1.30–3.79)
p trend			0.0009

[1]Adjusted for age, education, race, tubal ligation, family history of breast/ovarian cancer, menopausal status, use of oral contraceptives, parity and talc use.–[2]Included participants who started medication within 2 years of diagnosis/reference date.–[3]Additional adjustment for history of PID, gonorrhea, ovarian cysts, endometriosis, and fibroids. The RRs for aspirin, acetaminophen and other NSAIDs were mutually adjusted. Aspirin included regular aspirin, buffered aspirin; acetaminophen included Tylenol, coricidin, Dristan, darvocet, Percocet, Excedrin; other NSAID included advil, nurin, clinoril, motrin, anaprox, feldene, indocin, naprosyn.

association with NSAIDs use, one reported significant risk reduction with acetaminophen use[14] while 4 studies found significant reduced risk with use of other NSAIDs[15–18] but there were differences in these results. In one study, an inverse association was found only in nulliparous and nonoral contraceptive users.[18] No dose-response relationship was observed in a second study,[15] and information on NSAID use was limited to the 5 years before diagnosis in a third study.[17] Aspirin use was not significantly associated with risk in these 5 studies.[14–17,19] Ascertainment of NSAID use was heterogeneous in these studies: different NSAIDs were included, the exposure period varied (*e.g.*, adult use, use in previous 20 years or previous 5 years before ovarian cancer diagnosis), and information on frequency and duration of NSAID use was asked in only some studies.

An advantage of our study is that we collected detailed information on adult usage history of both over the counter and prescription NSAIDs including duration and frequency of use and indication for use. Our results suggest increased risk associated with duration and frequency of use of aspirin, acetaminophen and other NSAIDs although only the findings for other NSAIDs were statistically significant on their own. We adjusted for potential confounders and indication for use; the latter was considered in only some previous studies. Nevertheless, our results should be interpreted with caution for the following reasons. Our assessment of NSAID use was based on self-report without assessment of reliability of recall. However, a drug validation study conducted by colleagues in Los Angeles County found high and comparable concordance rate of recall of analgesics in cancer patients and control subjects.[20] Regular NSAID use was reported by 29% (31% in non-Hispanic whites) of controls in our study; comparable with the rate reported in Wisconsin and Massachusetts (34%)[18] but lower than that in Seattle (41%).[3] Differences in the assessment of use of NSAID complicate comparison of prevalences of use between studies.

Although an increased risk was specific to NSAIDs use and no increased risk was found with diuretic use, we cannot rule out the possibility of selective recall bias among ovarian cancer cases. Given that many NSAIDs products are available and use may be episodic, it is conceivable that some cases may be more motivated to remember their NSAID use than control subjects. There is also the possibility of surveillance bias and that certain health conditions led to regular NSAID use, resulting in frequent doctor visits, which increased the chances of ovarian cancer detection. As noted earlier, the prevalence of NSAID use was higher in women with LMP tumors or localized cancer than those with advanced stage cancers, and the magnitude of association was stronger for earlier stage cancers. However, the proportion of LMP/localized stage cancers among those we interviewed (41%) and those we failed to interview (39%) was not dissimilar, suggesting there should be minimal overestimation of the overall effect of NSAID in relation to this reason. There also may be residual confounding by indication for use. Another possible explanation for our observed positive finding is that women with early symptoms of undiagnosed ovarian cancer take pain medications to relieve these symptoms. This seems less likely because our results were essentially unchanged when we excluded participants who first started using these medications within the 5 years of diagnosis. Finally, we consider possibly that selection bias of cases and controls may have affected our finding. Our response rate was modest; cases who participated may differ from those who did not participate. Although controls in our study had more years of education than cases, there was no consistent pattern in the NSAID-risk association by education. The NSAID-risk association was most apparent in women who were college graduates but was very similar in women with high school education or less and those who had more than college education. Thus, despite these limitations, our results raise the concern that NSAIDs, taken as aspirin, acetaminophen or other NSAIDs, may actually increase the risk of ovarian cancer.

In our study, history of self-reported history of endometriosis that was diagnosed by a physician was associated with a significant 66% increased risk of ovarian cancer. Given that the elevated risk was observed for those with previous endometriosis for at least 11+ years, it is unlikely that our finding is due to detection bias but suggests that endometriosis may have an etiological role.

TABLE V – PREVALENCE OF NSAID USE IN CASES AND CONTROLS AND RRS (95% CI)[1] PER 5 YEARS OF NSAID USE

		ever NSAID-cases (%)	ever NSAID-controls (%)	10+ yrs of NSAIDs-cases (%)	10+ yrs of NSAID-controls	RR (95% CI) per 5 years of NSAID
Race/ethnicity	Non-Hipsanic Whites	45%	31%	17%	10%	1.23 (1.07–1.42)
	Other	31%	19%	7%	4%	1.37 (1.03–1.84)
Education	<College	36%	29%	14%	10%	1.09 (0.84–1.41)
	College graduate	48%	27%	16%	7%	1.71 (1.36–2.15)
	Graduate	33%	28%	11%	9%	1.05 (0.85–1.30)
Menopause	Premenopause	34%	22%	7%	5%	1.35 (1.05–1.73)
	Postmenopause	43%	34%	17%	11%	1.22 (1.06–1.42)
Tumor stage	LMP	47%	28%	14%	8%	1.37 (1.11–1.69)
	Invasive, Stage 1 or 2	40%	28%	14%	8%	1.36 (1.11–1.67)
	Invasive, Stage ≥3	37%	28%	13%	8%	1.16 (1.01–1.35)
Endometriosis	No	39%	27%	12%	9%	1.23 (1.08–1.40)
	Yes	50%	44%	26%	8%	1.52 (0.96–2.43)
Talc	No	36%	25%	13%	6%	1.31 (1.10–1.55)
	Yes	45%	35%	15%	14%	1.14 (0.94–1.38)
Oral Contraceptives	No	35%	25%	13%	10%	1.10 (0.89–1.37)
	Yes	43%	29%	14%	8%	1.31 (1.12–1.53)
Parity	No	41%	31%	15%	9%	1.28 (0.98–1.68)
	Yes	40%	27%	13%	8%	1.25 (1.09–1.45)
Pap smear[2]	≤5 times	34%	24%	11%	7%	1.22 (0.94–1.59)
	>5 times	42%	30%	15%	9%	1.27 (1.09–1.47)

[1]Adjusted for age, education, race, tubal ligation, family history of breast/ovarian cancer, menopausal status, use of oral contraceptives and parity.–[2]Frequency of Pap smears in the 10 years before reference date.

No association between endometriosis and ovarian cancer was reported in the Iowa Women's Health Study, but this may be because of the relatively limited number of ovarian cancers in this cohort and the low prevalence of endometriosis (~3%).[21] Endometriosis was associated with about a 30% increased risk in an Australian population-based case-control study[2] and in a pooled analysis of 2,098 cases and 2,953 controls from 4 US population-based case-control studies.[22] Although the prevalences of endometriosis among cases (8.4%) and controls (5.4%) in our study are very comparable with the figures reported in cases (8%) and controls (6%) in previous case-control studies,[2,22] a limitation of our study and other case-control studies on this topic is that history of endometriosis is not validated. We did not see meaningful differences in history of endometriosis by cell type (11% for endometrioid/clear cell *vs.* 8% for other cell types) of ovarian cancer while a higher prevalence of endometriosis in women with endometroid/clear cell has been usually reported iu other studies.[2,23] Interestingly, when one of us (CT) reviewed the pathology reports of the 52 ovarian cancer patients who reported a history of endometriosis, endometriosis in the ovary was documented in only 15 patients (15 of 604 cases = 2.5%) but the percent was higher in women with clear cell/endometrioid (7 of 84 = 8.3%) ovarian cancer compared with the other cell types (8 of 520 = 2.3%). Additional information on the type of endometriosis and location of endometriosis would be helpful in future studies.

The role of talc in the development of ovarian cancer has been studied extensively. In a 2006 review by the International Agency for Research on Cancer (IARC), talc was classified as possibly carcinogenic to humans (*i.e.*, Group 2B) on the basis that most of the 20 epidemiological studies on talc and ovarian cancer show consistently a 30–60% increased risk associated with talc use.[24] However, only about half of the studies examined exposure-response relationships and the evidence for this is less consistent. Our study adds to the small group of studies that have investigated the combination of frequency and duration of talc use on ovarian cancer risk.[25–28] Our results show a significant trend with increasing number of total applications. Using a combined index of total applications or cumulative lifetime days of talc use, 2 studies showed a higher risk with greater exposure[27,29] but this was not observed in 2 other studies.[25,28] When we investigated the combined effect of frequency and duration, our results suggest that the effect of increasing frequency was modest in users of less than 20 years but that the effect of frequency was clearer in women who had used talc for 20 years or more. Our results also suggest that talc use prior to 1976 may be more important. In 1976, talcum powder manufacturers instituted voluntary guidelines to prevent asbestos contamination in talc products and thus formulations after 1976 may be less likely to be contaminated with asbestos fibers. Stronger associations with talc use in the 1960s and 1970s have been reported in some studies[25,27] but not in others.[2,28] Thus, lack of sufficient information on frequency, duration and calendar period of talc use may have contributed to misclassification of this exposure variable in some previous studies.

Our findings on talc use and endometriosis and ovarian cancer risk are compatible with previous studies. However, the NSAID finding in this study was unexpected and requires confirmation with further characterization of the association by frequency and duration of use, cumulative dose and timing of exposure. In addition, it will be important to evaluate the underlying conditions for medication use.

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Exhibit 59

Systematic Review and Meta-Analysis of the Association between Perineal Use of Talc and Risk of Ovarian Cancer

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Abstract

Over the past four decades, there has been increasing concern that perineal use of talc powder, a commonly used personal care product, might be associated with an increased risk of ovarian cancer.

Objectives: To systematically review all available human epidemiological data on the relationship between perineal use of talc powder and ovarian cancer, with consideration of other relevant experimental evidence.

Methodology: We identified 30 human studies for qualitative assessment of evidence, including 27 that were retained for further quantitative analysis.

Results: A positive association between perineal use of talc powder and ovarian cancer was found [OR: 1.28 (95% CI: 1.20 - 1.37)]. A significant risk was noted in Hispanics and Whites, in women applying talc to underwear, in pre-menopausal women and in post-menopausal women receiving hormonal therapy. A negative association was noted with tubal ligation.

Conclusion: Perineal use of talc powder is a possible cause of human ovarian cancer.

Keywords: Talc; ovarian cancer; perineal; epidemiological studies; systematic review; meta-analysis; toxicological studies.

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1. Introduction

Ovarian cancer is a common gynecologic cancer among women in developed countries, occurring at low rates among young women but increasing with age [1]. The annual incidence rate of ovarian cancer during the period 2005 – 2009 was 12.7/100,000 women, varying by ethnicity. The majority of ovarian cancers are diagnosed at an advanced stage, with 61% having distant metastases at diagnosis. Hereditary risk factors for ovarian cancer, specifically BRCA1 gene mutations, increase the risk above 35 years of age by about 2-3%.

In recent decades, there has been increasing concern that perineal exposure to talc, a commonly used personal care product, might be associated with an increased risk of ovarian cancer. However, the data describing this association is somewhat inconsistent. Perineal application of talc among women varies by geographic location (Supplementary Material I), with prevalence of use generally higher in Canada, the US and the UK compared to Greece, China and Israel [2].

In order to better characterize the potential ovarian cancer risk associated with perineal use of talc, we conducted a systematic review and meta-analysis of peer-reviewed human studies on this issue. We also examined additional in-vitro or in-vivo toxicological studies, which shed light on possible biological mechanisms that might support an association between and ovarian cancer.

2. Materials and Methods

2.1. Literature Search and Identification of Relevant Human Studies

A comprehensive, multi-step search strategy was used to to identify relevant studies on talc from multiple bibliographic databases, relevant national and international agencies and other grey literature sources (Supplementary Material II). Specifically, conducted a systematic search for all original studies involving human subjects that examined the association of genital/perineal use of talc powder and risk of ovarian cancer, including studies identified in a previous review by Berge et al. [3]. This review followed the PRISMA guidelines, and more specific guidance provided by the Cochrane Collaboration [4] (see Supplementary Material II for details).

Included studies were individually evaluated and scored by two reviewers (MT and NF), as detailed in the Table 1 and Supplementary Material XI. Studies included in previous reviews by both Berge et al. [3] and Penninkilampi et al [5] are compared in Supplementary Material I.

The quality of included studies was assessed using the Newcastle-Ottawa Scale (NOS) [6], as detailed in Supplementary Material IV. We used a cut-off point of 7+ stars to represent studies of higher quality.

2.2. Literature Search and Identification of Relevant Non-Human Studies

We conducted a (non-systematic) review of relevant non-human studies identified in three major bibliographic databases to identify potentially relevant animal

and in vitro studies (Supplementary Material V). Only studies that focused on perineal exposure to talc powder were included. For outcomes, studies that focused on any type of cancer including ovarian cancer and perineal exposure were considered. All retrieved studies were examined for relevance, reliability and overall quality using the Klimisch scoring system [7, 8] (Supplementary Material VII, VIII and IX).

Studies are classified into one of the following four categories of reliability: 1) reliable without restriction, 2) reliable with restrictions, 3) not reliable and 4) not assignable. Additionally, category (5) is assigned to special studies focusing on pharmacologic or mechanistic investigations.

2.3. Hazard Characterization

Epidemiological studies included in the systematic review were qualitatively assessed to examine their potential to inform a weight of evidence analysis. Findings from these studies were evaluated with respect to study design, exposure and outcome ascertainment, as well as potential sources of bias and confounding.

Animal studies were evaluated for evidence on the association between perineal application of talc and ovarian cancer. Additional information on mechanism of action and toxicokinetics derived from in-vitro and in-vivo studies was used in evaluating biological plausibility.

We evaluated the overall weight of scientific evidence by performing a qualitative evaluation of the findings collected from epidemiological studies as well as non-human studies, using the Hill criteria [9].

2.4. Quantitative Meta-Analysis

We conducted a meta-analysis of the risk of ovarian cancer in relation to perineal use of talc using quantitative risk estimates reported in 27 original studies, comprising three cohort studies and twenty-four case-control studies (included in Table 1). Studies that had analyzed overlapping study populations were assessed on a case-by-case basis for inclusion into the meta-analysis. The level of detail in the reported findings, including sample size and publication date, were considered when deciding which study to include in the case of overlap (Supplementary Material XIV).

Maximally adjusted odds ratios (ORs), hazard ratios (HRs) or relative risks (RRs) – measures that are largely comparable because of the relatively low rate of occurrence of ovariaion cancer – were extracted from the original studies. Details of the meta-analytic methods are provided in Supplementary Material XIV.

114 **Table 1: Characteristics and overall findings of all included studies (N=30).**

Study (Location)	Sample Size (Cases/ Controls or Cases/ Total Cohort)	Age (Years)	Subgroup Analyses	Exposure-Response Assessment	Overall Author Conclusion	NOS[1]
Case-control studies						
Booth et al.* (1989), UK [10]	235/451	Range: 20-65 Mean: 52.4 (cases); 51.4 (controls)	Frequency	No trend found	Possible association with >weekly use.	5
Chang and Risch (1997), Canada [11]	450/564	Range: 35-79 Mean: 57.2 (cases); 57.5 (controls)	Ever use Frequency Duration Time of use Type of use	Possible exposure-response with frequency and duration of use	Positive association	7

[1] Newcastle-Ottawa Scale (NOS) score for each of the listed studies as assessed in our review

Study (Location)	Sample Size (Cases/ Controls or Cases/ Total Cohort)	Age (Years)	Subgroup Analyses	Exposure-Response Assessment	Overall Author Conclusion	NOS[1]
			Pelvic surgery Histology			
Chen et al.* (1992), China [12]	112/224	Mean: 48.5 (cases); 49.0 (controls)	Ever use;	No trend analysis conducted	Positive association with use >3 months	6
Cook et al. (1997), USA [13]	313/422	Range: 20-79	Ever use Duration Type of use Histology Lifetime applications	No trend found	Positive association.	7
Cramer et al. (1982), USA [14]	215/215	Range: 18-80 Mean ± SD: 53.2 ± 1.0 (cases); 53.5 ± 1.0 (controls)	Ever use Type of use Pelvic surgery	No trend analysis conducted	Positive association	6

Study (Location)	Sample Size (Cases/ Controls or Cases/ Total Cohort)	Age (Years)	Subgroup Analyses	Exposure-Response Assessment	Overall Author Conclusion	NOS[1]
Cramer et al. (2016), USA [15]	2,041/2,100	Range: 18-80	Ever use; Frequency; Duration; Type of use; Histology; Type of powder; Pelvic surgery; Ethnicity; Age at first use; Time since last exposure;	Significant trend for years since exposure, frequency and duration of use, and number of lifetime applications	Positive association	7
Gates et al. (2008), USA [16]	New England Case Control (NECC): 1,175/1,202 Nurses' Health	Mean ± SD: 51 ±13 (NECC); Mean ± SD: 51 ±8 (NHS)	Ever use; Frequency;	Significant trend for frequency of use	Positive association	7

Study (Location)	Sample Size (Cases/ Controls or Cases/ Total Cohort)	Age (Years)	Subgroup Analyses	Exposure-Response Assessment	Overall Author Conclusion	NOS[1]
	Study (NHS): 210/600					
Godard et al. (1998), Canada [17]	153/152	Mean: 53.7	Ever use; Sporadic/familial	No trend analysis conducted	No association	5
Green et al. (1997), Australia [18]	824/860	Range: 18-79	Ever use; Pelvic surgery;	No trend found	Positive association	7
Harlow et al. (1989), USA [19]	116/158	Range: 20-79	Ever use; Type of use; Type of powder;	No trend analysis conducted	No association	7
Harlow et al. (1992), USA [20]	235/239	Range: 18-76	Ever use; Frequency; Duration; Type of use;	Significant trend for monthly frequency of use	Positive associations in certain subgroups (talc used before 1960, women <50	7

Study (Location)	Sample Size (Cases/ Controls or Cases/ Total Cohort)	Age (Years)	Subgroup Analyses	Exposure-Response Assessment	Overall Author Conclusion	NOS[1]
			Method of use; Histology; Tumor grade; Type of powder; Lifetime applications; Age of first use; Pelvic surgery;		years old, women with 1 or 2 live births)	
Hartge et al. (1983), USA [21]	135/171	Mean: 52.1 (cases); 52.2 (controls)	Ever use;	No trend analysis conducted	No association	5
Kurta et al. (2012), USA [22]	902/1,802	Range: No range reported (age 25+)	Ever use;	No trend analysis conducted	Positive association	6
Langseth & Kjaerheim (2004), Norway [23]	46/179	Not reported	Ever use,	No trend analysis conducted	No association	4

Study (Location)	Sample Size (Cases/ Controls or Cases/ Total Cohort)	Age (Years)	Subgroup Analyses	Exposure-Response Assessment	Overall Author Conclusion	NOS[1]
Merritt et al. (2008), Australia [24]	1,576/1,509	Range: 18-79 Mean: 57.8 (cases); 56.4 (controls)	Ever use; Duration; Histology; Pelvic surgery; Age at diagnosis;	No trend found	Positive association strongest for serous and endometrioid subtypes.	7
Mills et al. (2004), USA [25]	249/1,105	Mean ± SD: 56.6 (cases); 55 (controls)	Ever use; Frequency; Duration; Year of first use; Histology; Pelvic surgery; Time of use; Tumor behavior; Cumulative use;	No trend found	Positive association for invasive and serous invasive tumors.	6

Study (Location)	Sample Size (Cases/ Controls or Cases/ Total Cohort)	Age (Years)	Subgroup Analyses	Exposure-Response Assessment	Overall Author Conclusion	NOS[1]
Moorman et al. (2009), USA [26]	African-American: 143/189; White 943/868	Range: 20-74	Ever use; Ethnicity;	No trend analysis conducted	No association	6
Ness et al. (2000), USA [27]	767/1,367	Range: 20-69	Ever use; Duration; Method of use;	No trend found	Positive association for any method of use.	6
Rosenblatt et al. (1992), USA [28]	77/46 (analyzed)	Range: ≤30 – 80≥	Ever use; Duration; Type of use; Pelvic surgery;	Positive trend for duration of use since tubal ligation	Possible association	4
Rosenblatt et al. (2011), USA [29]	812/1,313	Range: 35-74	Ever use; Lifetime number of applications; Duration;	No trend found	Possible association	7

Study (Location)	Sample Size (Cases/ Controls or Cases/ Total Cohort)	Age (Years)	Subgroup Analyses	Exposure-Response Assessment	Overall Author Conclusion	NOS[1]
			Year of first use; Age of first use; Age of last use; Time of use; Type of use; Histology;			
Schildkraut et al. (2016), USA [30]	584/745	Range: 20-79	Ever use; Frequency; Duration; Histology; Lifetime applications; Menopausal status;	Significant trend with frequency and duration of use, and number of lifetime applications	Positive association	8
Tzonou et al. (1993), Greece [31]	189/200	Range: <70	Ever use;	No trend analysis conducted	No association	5

Study (Location)	Sample Size (Cases/ Controls or Cases/ Total Cohort)	Age (Years)	Subgroup Analyses	Exposure-Response Assessment	Overall Author Conclusion	NOS[1]
Whittemore et al. (1988), USA [32]	188/539	Range: 18-74	Ever use; Frequency; Duration; Type of use; Pelvic surgery;	No trend found	Could neither implicate nor exonerate talc as an ovarian carcinogen	4
Wong et al. (1999, 2009), USA [33, 34]	462/693	Mean: 54.9	Ever use; Type of use; Duration; Pelvic surgery;	No trend found	No association	4
Wu et al. (2015), USA [35]	1,701/2,391	Range: 18-79	Ever use; Ethnicity;	No trend analysis conducted	Positive association among Hispanics and non-Hispanic whites, but not African Americans.	7

Study (Location)	Sample Size (Cases/ Controls or Cases/ Total Cohort)	Age (Years)	Subgroup Analyses	Exposure-Response Assessment	Overall Author Conclusion	NOS[1]
Wu et al. (2009), USA [34]	609/688	Range: 18-74	Ever use; Frequency; Duration; Type of use; Histology; Time of use; Cancer stage;	Significant trend for frequency and duration of use, and number of lifetime applications	Positive association	7
Cohort studies						
Gates et al. (2010)*, USA [36]	797/108,870	Range: 30-55	≥/week vs <1/week; Histology;	No trend analysis conducted	Possible association that varies by histological subtype. No association with mucinous tumors.	7

Study (Location)	Sample Size (Cases/ Controls or Cases/ Total Cohort)	Age (Years)	Subgroup Analyses	Exposure-Response Assessment	Overall Author Conclusion	NOS[1]
Gertig et al. (2000), USA [37]	307/78,630	Range: 30-55 (at cohort entry)	Ever use; Frequency; Histology; Race;	No trend found	Possible association (modest increase for serous invasive subtype)	5
Gonzalez et al. (2016), USA [38]	154/41,654	Range: 35-74 Median: 57.8	Ever use; Time of use;	No trend analysis conducted	No association	6
Houghton et al. (2014), USA [39]	429/61,285	Range: 50-79 Mean: 63.3	Ever use; Duration; Type of use; Histology;	No trend found	No association	7

** Study assessed for qualitative evidence but not included in the meta-analysis*

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3. Results

3.1. Evidence from Human Studies

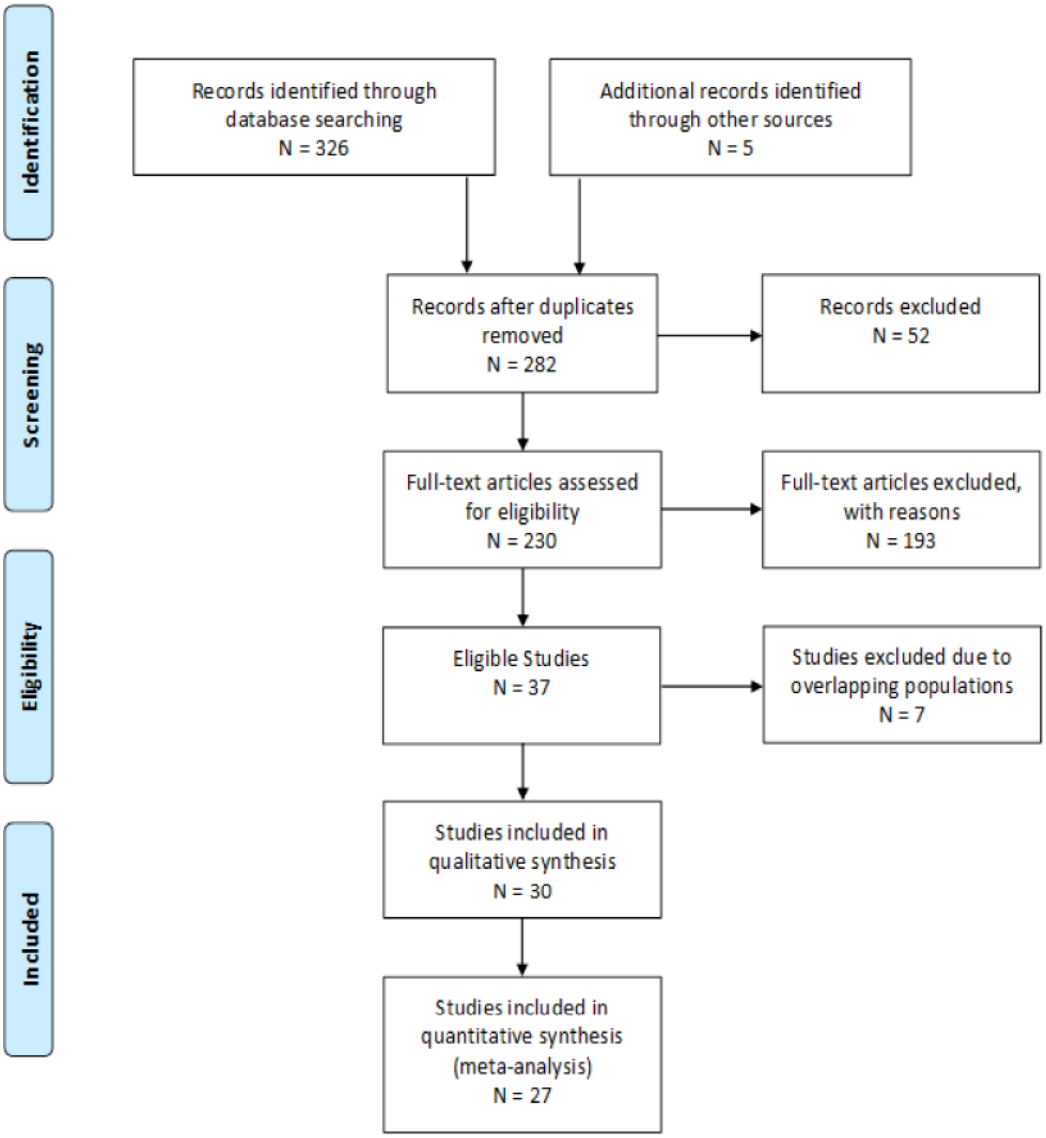
The multiple database search for original human studies yielded 656 references. Although grey literature search yielded another 477 references, only 5 were judged relevant the present analysis. Automatic followed by manual removal of duplicates identified 282 references for screening and review.

Multi-level screening and full-text examination resulted in the in the inclusion of 30 studies for further qualitative/quantitative analyses (Supplementary Materials X and XI). A detailed PRISMA flow diagram is shown in Figure 1 [40]. Key characteristics of the included 26 case-control studies and four cohort studies are summarized in Table 1.

Twenty-one of the thirty studies were carried out in the USA, with the remaining studies conducted in Europe (n=4), Canada (n=2), Australia (n=2) and China (n=1). Forty percent (n=12) of the studies were relatively recent, published in the last decade, with the remaining studies published between 1982 and 2006. The study populations generally included adult women. Several studies analyzed data from populations initially recruited for other purposes, such as the Nurses' Health Study (NHS) [15, 36, 37] and Women's Health Initiative (WHI) [39].

The number of ovarian cancer patients analyzed varied from as few as 46 cases [23] to 22,041 cases [15]. Twenty-seven out of the 30 included studies assessed the association between ever use of perineal talc use and ovarian cancer. Subgroup

136 analyses examining the effect of frequency and duration of use, type of use, period of
137 use and other factors varied among these studies (Table 1).



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139 Figure 1: PRISMA Flow Diagram

140 Sixty three percent (n=19) of the studies concluded the presence of a positive
141 association between perineal exposure to talc powder and ovarian cancer risk [10-16,

18, 20, 22, 24, 25, 27-30, 34-36]. Ten studies concluded the absence of an association [17, 19, 21, 23, 26, 31, 33, 37-39]. Only one study could not reach a clear conclusion on the presence or absence of an association [32]. Many of the included studies reported variability in some of the analyzed subgroups regarding possible association between exposure to talk powder and risk of ovarian cancer. Supplementary Material X presents the findings and details of all the studies included in the analysis, while Supplementary Material XI summarizes the strengths and limitations of each of these studies as identified by the original study authors and by us.

3.2. Evidence from Non-Human studies

After removal of duplicates, the bibliographic database searches on non-human studies initially yielded 1,165 references. The 51 retained animal studies focusing on the carcinogenicity of talc, mechanism of action, and toxicokinetics are summarized in Supplementary Material XII.

3.3. Hazard Characterization

3.3.1. Evidence from Human Studies

The case-control studies generally included adult women participants. Cases were commonly selected from registries or hospital records, and included all eligible subjects within a specific geographic region and diagnosed with ovarian cancer within a predetermined time period. Controls were generally matched to cases by age and residence. All the included studies compared the risk of ovarian cancer in ever vs never

users of talc (perineal application). However, several of the studies also included subgroup analyses to examine the potential effect of frequency of use, duration of use, tumor histology, ethnicity, method of use, lifetime number of applications, year of first use, and menopausal status. Some authors concluded that the risk of ovarian cancer is limited to [or stronger in] certain subgroups (weekly talc users, premenopausal women) or for specific histology types (notably serous tumors).

Studies reported effect estimates adjusted for a variety of potential confounders (see detailed tables in Supplementary Material X & XI). Age and parity were considered the two most important variables that could introduce potential bias, based on prior literature: few studies reported findings that were not adjusted for these two variables. As many of the studies only reported on the ovarian cancer risk assessing only one exposure category (comparing only ever vs never users of talc), exposure-response analyses were not done in all studies. When conducted, findings from trend analyses were not consistent.

3.3.2. Evidence from Non-Human Studies

The following aspects were considered in the weight of evidence assessment of ovarian cancer and perineal exposure to talc:

- hazards arising from the physical and chemical properties of talc, including potential structure-activity relationship indicative of carcinogenic potential;
- the toxicokinetics of talc and the ability to migrate from the perineal area to ovaries and quantity at the actual target site (the tissue dose);

- evidence on ovarian cancer reported in animal studies; and
- findings from in vitro studies suggestive of mechanism of action of carcinogenic effect.

While the data from the animal studies considered various routes of talc administration are inconsistent [41-46], there are observations from in vivo and in vitro studies which support the potential for local carcinogenic action of talc on fallopian, ovarian and peritoneal epithelium [27, 47-53].

The results from the *in vitro* studies are informative for mechanisms of action of possible carcinogenicity. Smith and colleagues [54] identified 10 key characteristics (KCs) commonly exhibited by established human carcinogens.

Oxidative stress (KC 6) and inflammation (KC 5) in cell cultures induced by talc have been reported by several authors [48], corresponding to two of the 10 key characteristics (KCs) described by Smith et al. [54]. Several authors suggested additional potential mechanisms of action through cell proliferation (KC 10) and changes in gene expression, presumably facilitated by oxidative stress and dysregulated antioxidant defense mechanisms [49, 55].

Chronic perineal or vaginal exposures of animals to talc do not directly affect ovulation or steroidal hormone levels, but can induce chronic local inflammation, which has been suggested as a risk factor for ovarian cancer [56]. Mechanism of action studies suggested that talc can complex iron on the surface and disrupt iron homeostasis, associated with oxidant generation, macrophage distress and leukotriene

released by macrophages in the surrounding cells resulting in the inflammatory response which could act as a tumor promoter in both animals and humans [48, 50, 51].

The changes seen in cultured cells after exposure to talc [50, 51] are consistent with those inflammatory and proliferative processes in the lungs seen in laboratory animals after inhalation exposure in a 1993 study conducted by the US National Toxicology Program [47]. In female rats, hyperplasia of alveolar epithelium was associated with inflammatory response and occurred in or near foci of inflammation [47]. The severity of the fibrous granulomatous inflammation in the lungs increased with increased talc concentrations and exposure duration and a significant association was observed between inflammation and fibrosis in the lungs and the incidence of pheochromocytomas in this study [47]. Overall, the avaialbe experimental data suggest irritation, followed by oxidative stress and inflammation, may play be involved in local carcinogenic effects of talc in the ovaries.

Local inflammation of the epithelial ovarian surface in rats following by injection of a suspension of talc particles demonstrated the development of foreign body granulomas surrounding talc particles and large ovarian bursal cysts [53]. It is generally accepted that benign and malignant ovarian epithelial tumors arise from surface epithelium and its cystic derivatives, and surface epithelial cysts have a greater propensity to undergo neoplasia than does the surface epithelium itself [57]. Evidence of neoplasms of epithelial origin, nuclear atypia, or mitotic activity in the surface epithelium was not found in this study; however, focal areas of papillary changes in the surface epithelium consistent with the histological signs of premalignancy were observed in 40% of treated animals [53].

Data on talc migration in the genital tract of animals is inconsistent, but could not exclude such possibility [58-61]. Some studies have reported lack of neutron-activated talc migration from the vagina to the ovaries in cynomolgus monkeys [58], but talc particles were identified in the ovaries of rats that received intrauterine instillation of talc [60]. Radioactivity was not found in the ovaries of rabbits dosed intravaginally with tritium-labelled talc, but was detected in cervix and fallopian tubes [59-61]. In studies in humans, Henderson and colleagues [62] examined tumor tissue of female patients with ovarian and cervical tumors. The authors detected talc particles in histological samples from 10 of 13 ovarian tumors, 12 of 21 cervical tumors and in 5 samples of 12 normal ovarian tissues [62].

Historically, the concern for talc carcinogenicity has been associated with its contamination by asbestos fibers (tremolite) [63], which is considered carcinogenic to humans [2]. Talc, including baby powder, available in the US, contains only U.S. Pharmacopeia (USP) grade pure talc [64]. Talcum powder has been asbestos-free since the 1976 where the specifications for cosmetic talc were developed [65].

3.3.3. Weight of evidence for carcinogenicity

Based on our evaluation of the weight of multiple lines of evidence, we concluded that perineal application of talc is a possible casue of cancer ovarian cancer in humans. In 2010 the Internatinal Agency for Research on Cancer [2] categorized perineal use of talc-based body powder (not containing asbestos or asbestiform fibers) as “possibly carcinogenic to humans (Group 2B)” [66].

Table 2 summarizes the available evidence for the association of ovarian cancer with perineal application of talc, organized around the nine Hill criteria [9]. Additoinal details of this evaluation are given in Supplementary Material XIII.

Table 2: Summary of evidence for each of the Hill Criteria of causation, as applied to perineal application of talc and ovarian cancer

Criterion	Summary of Evidence
Strength of association	• Out of the 30 epidemiological studies, six reported positive association of statistical significance with a risk value (relative risk or odds ratio) of 1.5 or greater • None of the cohort studies (n=3) found statistically significant association
Consistency	Fifteen out of thirty studies reported positive and significant associations reported in: • Different ethnicities (Caucasians, African Americans, and Latin Americans); • Over four decades (1982 - 2016); • Mostly in studies from the United States but also in other countries (Canada, Australia and China) • Case-control studies but not in cohort studies
Specificity	• Overall, the perineal talc exposure is specifically associated with cancer of the ovary and not other organs • No evidence of other target organs (e.g., liver) being associated with perineal application of talc (via systemic exposure)

Criterion	Summary of Evidence
	• Thirteen studies included analyses by histologic type of ovarian cancer, and eight of them found a significant increase in the risk of serous ovarian cancer in talc users
Temporality	• In all case-control studies reporting positive outcome, the participants recalled that exposure to talc preceded the reported outcome • In cohort studies, the follow up period could have been inadequate (<15 years) to detect a potential association between talc exposure and ovarian cancer
Biological gradient (exposure-response)	• About half of the epidemiological studies assessed only one level of talc exposure (ever vs never usage) • Of the 12 studies reporting a positive association, six studies found significant exposure-response trend, particularly with medium and high frequency usage groups Regarding duration of use/exposure to talc, several studies reported the greatest risk in the 20+ years of use exposure group, followed by the 10-20 years' group, then the <10 years' group
Biological plausibility	• Particles of talc appear to migrate into the pelvis and ovarian tissue causing irritation and inflammation • Transport of talc via perineal stroma and presence in ovaries documented • Chronic inflammatory response and alteration in local immunogenicity are possible mechanisms
Coherence	• Results from talc epidemiology studies are coherent with the current knowledge on the risk factors for ovarian cancer (e.g., factors/physiological states associated with greater frequency and duration of ovulation are associated with increased risk of ovarian cancer)

Criterion	Summary of Evidence
	• Many (but not all) case-control studies reported lower risk of ovarian cancer in women who underwent pelvic surgery or tubal ligation (which disrupts the pathway and movement of talc from lower to upper genital tract) & suppressed ovulation
Experimental evidence	• Perineal application of talc has not been tested in an animal model of ovarian cancer • The single animal cancer bioassay with talc conducted by the US National Toxicology Program was only by the inhalation route • Rodent models may be of limited relevance because of ovulations occurring only or mainly during the breeding season and the rarity of ovarian epithelial tumors in these animals and ovaries are variously enclosed in an ovarian bursa.
Analogy	• Talc and asbestos are both silicate minerals • Talc has been variably contaminated with asbestos (tremolite and anthophyllite; until 1976, talcum powders were only required to contain at least 90% mineral talc) • The pleural and peritoneal mesotheliomas caused by asbestos are histologically similar to epithelial ovarian cancer associated with talc • In animal models, asbestos induces ovarian epithelial hyperplasia similar to early epithelial tumors reported in women with past use of talc

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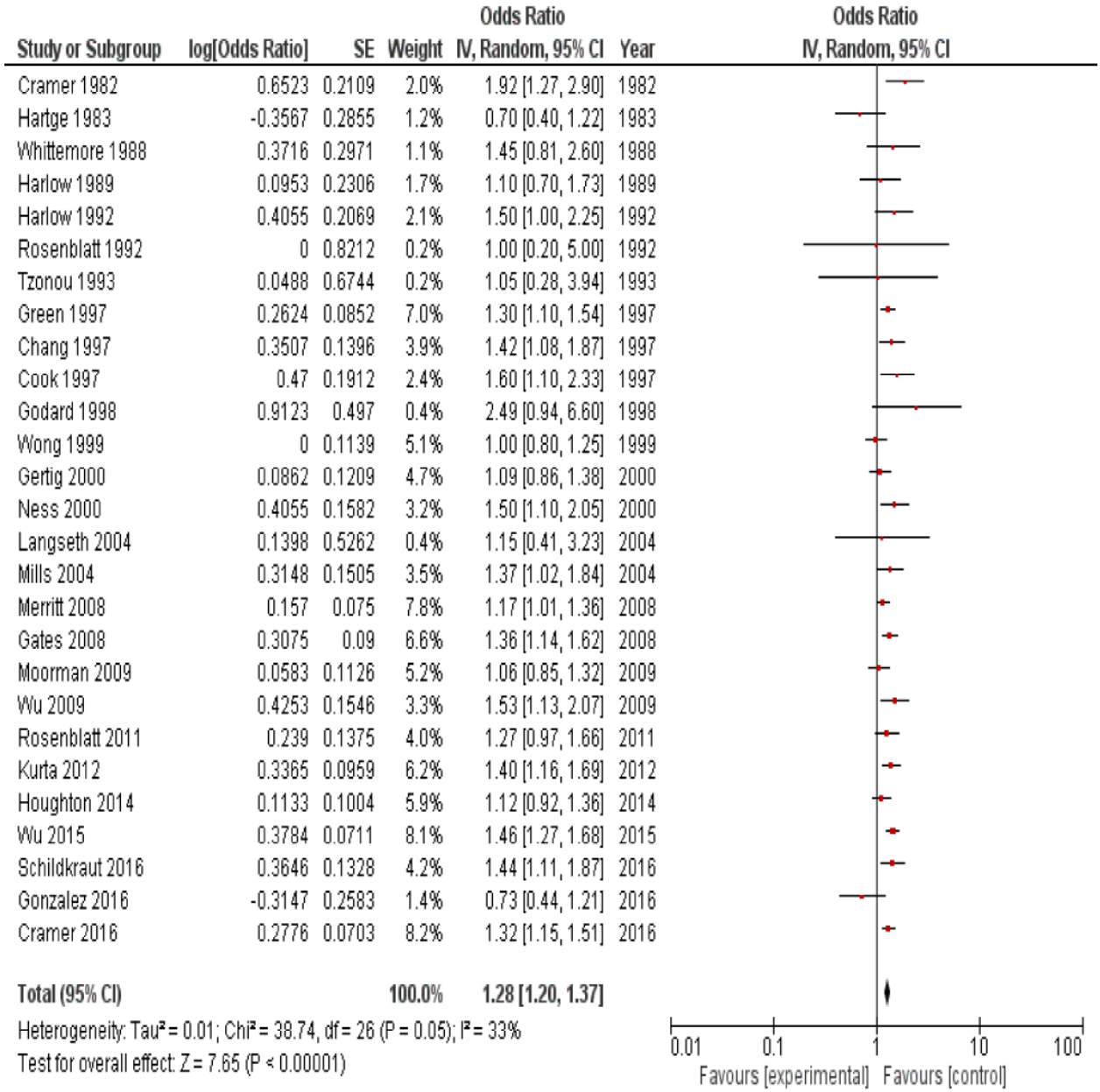
3.4. Meta-Analysis

The use of genital talc was associated with a significant increase in the risk of epithelial ovarian cancer, with an overall odds ratio [OR] based on our meta-analysis of 1.28 (95% confidence interval [CI]: 1.20 to 1.37 P<0.0001, I^2= 33%), as presented in

265 Figure 2. This result is comparable to those of earlier meta-analyses conducted by other

266 investigators [3, 5, 67-69] as shown in Supplementary Material I.



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268 **FIGURE 2: Forest plot of the meta-analysis results on perineal use of talc and**

269 **risk of ovarian cancer**

An increased risk is more apparent in Hispanics and Whites, in women applying talc to underwear, in pre-menopausal women and post-menopausal women receiving hormonal therapy, as well as for the serous and endometrioid types of ovarian cancer (Table 3 and Supplementary Material XIV). A negative association was noted with tubal ligation. Our analysis pooled risk estimates from 27 original studies including 3 cohort studies and 24 case-control studies, spanning across four decades (1982-2016) and including a total of 16,352 cases and 19,808 controls from different ethnicities.

In assessing heterogeneity among included studies, most subgroup analyses reported an I^2 statistic ranging between 0%-40%, which will have only a minimal impact on the analysis [4]. Only three subgroup analyses (ethnicity, menopausal state, and pelvic surgery) reported an I^2 statistic of 77%-78%, where considerable heterogeneity might have had an impact on the results [4]. (See Table 3 and Supplementary Material XIV for a listing of I^2 statistic values for the different subgroup analyses)

Whereas case-control studies showed a significant increase in the risk of ovarian cancer for ever vs never users of talc powder [OR: 1.32 (95% CI: 1.24 to 1.40), P < 0.00001, I^2= 22%], cohort studies failed to show a significant increase in risk [OR: 1.06 (95% CI: 0.9 to 1.25), P= 0.49, I^2= 17%]. Thirteen out of 24 case-control studies (54%) showed a statistically significant association, whereas none of the 3 cohort studies showed a significant overall association between ever vs never genital talc exposure and risk of ovarian cancer.

Subgroup analysis by study quality (NOS≥7 vs NOS<7) did not show any significant differences in the overall pooled risk estimate. Similarly, there were no differences among subgroup analysis conducted by decade of publication. A significant association was observed for population-based studies [OR: 1.34 (95% CI: 1.27 to 1.41), P < 0.00001, I^2= 0%], but for enlisting hospital-based controls [OR: 0.96 (95% CI: 0.78 to 1.17), P= 0.66, I^2= 0%].

We conducted influence analysis to examine the impact of individual studies on the results of our meta-analysis. No appreciable changes were observed regarding the overall association of perineal talc exposure and the risk of ovarian cancer in response to the exclusion of any one study. Detailed results from the influence analysis are provided (Supplementary Material XIV).

Subgroup analysis based on ethnicity indicated that Hispanic women using talc showed the most significant increase in risk of ovarian cancer [OR: 1.70 (95% CI: 1.17 to 2.47), P = 0.005, I^2= 0%], followed by White women [OR: 1.28 (95% CI: 1.10 to 1.49], P= 0.001, I^2= 56%). African-American women showed a non-significant association with ovarian cancer in [OR: 1.67 (95% CI: 0.90 to 3.10), P= 0.1, I^2= 48%].

Analyzing exposure by frequency of talc use, talc exposure was stratified into three groups: high (once daily for >25 days/month), medium (once daily for 10–25 days/month) and low (once daily for 1–<10 days/month). The OR for the high-use group was higher in the high-use group compared to the other two groups (medium and low-use groups). Duration of talc use was stratified into three groups: <10 years, 10 – <20 years, and 20+ years. The overall odds ratio of the <10 years' group was lower than the

OR for the 10 – <20 years' group. On the other hand, the OR for the 20+ years' group was lower and not statistically significant. However, this OR was based on two studies that showed considerable heterogeneity (I^2=75%). Examining the method of application of talc, application to the underwear subgroup had a statistically significant OR, which was the highest among all subgroups. Diaphragm use showed an expected, yet non-significant, negative association with ovarian cancer, which may be due to its action blocking the ascent of talc particles up the reproductive tract.

Pooled risk estimates were statistically significant for two histological types of ovarian cancer: serous tumors [OR: 1.38 (95% CI: 1.22 to 1.56), P < 0.00001, I^2= 0%] and endometrioid tumors [OR: 1.39 (95% CI: 1.05 to 1.82), P= 0.03, I^2= 2%]. The mucinous type showed a non-significant association [OR: 1.05 (95% CI: 0.85 to 1.29), P= 0.41, I^2= 23%], while there were not sufficient studies to examine the other types of ovarian cancers. Regarding tumor behavior, there was no appreciable difference between invasive [OR: 1.38 (95% CI: 1.15 to 1.65), P= 0.0004, I^2= 0%] and borderline [OR: 1.43 (95% CI: 1.08 to 1.89), P= 0.01, I^2= 19%] grades of ovarian cancer. Borderline serous tumors showed slightly greater risk [OR: 1.39 (95% CI: 1.09 to 1.78), P= 0.008, I^2= 0%] compared to the serous invasive grade [OR: 1.32 (95% CI: 1.13 to 1.54), P= 0.0004, I^2= 24%], while both showed a significant association with perineal talc exposure. However, the mucinous tumors showed a non-significant association with talc exposure, with invasive grades being associated with a greater risk [OR: 1.34 (95% CI: 0.48 to 3.79), P= 0.58, I^2= 70%] compared to the borderline grade [OR: 1.18 (95% CI: 0.76 to 1.82), P < 0.46, I^2= 34%].

Among post-menopausal women, those receiving hormonal therapy showed the greatest risk [OR: 2.28 (95% CI: 1.72 to 3.01), P < 0.00001, I^2= 0%], followed by pre-menopausal women [OR: 1.42 (95% CI: 1.16 to 1.75), P= 0.0008, I^2= 0%], and then post-menopausal women not receiving hormonal therapy [OR: 1.05 (95% CI: 0.84 to 1.32), P= 0.66, I^2= 25%]. This subgroup analysis suggests that hormonal factors, especially estrogens influence the risk of developing ovarian cancer among postmenopausal women who have perineal talc exposure.

Women with prior ligation of the Fallopian tubes showed a significant reduction in risk [OR: 0.64 (95% CI: 0.45 to 0.92), P= 0.02, I^2= 19%] against ovarian cancer compared to hysterectomy [OR: 0.89 (95% CI: 0.54 to 1.46), P= 0.65, I^2= 61%], whereas both surgeries combined showed no effect [OR: 1.06 (95% CI: 0.78 to 1.42), P= 0.72, I^2= 61%]. This might be attributed to the fact that tubal ligation is usually performed at an earlier age, thus preventing entry of talc into the reproductive tract earlier and prolonged exposure to talc, compared to hysterectomy that is performed later in life where a higher exposure has already taken place. In a recent meta-analysis [70], the authors reported a negative association of tubal ligation (27 studies) and hysterectomy (15 studies) with the risk of ovarian cancer: this negative association was more apparent in women who had the surgery at an earlier age. A highly plausible mechanism for this association, as suggested by the authors, involves blocking of ascent of agents such as talc to the ovaries.

A summary of results of our meta-analysis is shown in Table 3. Forest plots of all sub-group analyses are provided in Supplementary Material XIV.

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359 **Table 3: Results of the subgroup analysis of talc exposure and ovarian cancer**

Outcome or Subgroup	Studies	Effect Estimate [95% CI)	Heterogeneity I^2 Statistic [p-value]
1. Talc use			
Ever vs. Never	27	1.28 [1.20, 1.37]	33% [< 0.00001]
Ethnicity	3		77% [0.08]
African Americans	3	1.67 [0.90, 3.10]	48% [0.10]
Hispanics	2	1.70 [1.17, 2.47]	0% [0.005]
Whites	3	1.28 [1.11, 1.49]	56% [0.001]
Asians	1	0.04 [0.01, 0.16]	N/A
2. Study Assessment			
2.1. Study Design	27		33% [< 0.00001]
Case-Control	24	1.32 [1.24, 1.40]	22% [< 0.00001]
Cohort	3	1.06 [0.90, 1.25]	17% [0.49]
2.2. Type of Controls	24		22% [< 0.00001]
Hospital-based	4	0.96 [0.78, 1.17]	0% [0.66]
Population-based	19	1.34 [1.27, 1.41]	0% [< 0.00001]
Combined	1	1.45 [0.81, 2.60]	N/A
2.3. Quality Score (NOS)	27		33% [< 0.00001]
NOS >=7	12	1.32 [1.25, 1.40]	0% [< 0.00001]
NOS <7	15	1.21 [1.05, 1.39]	47% [0.009]
2.4. Publication Year	27		33% [< 0.00001]
1980-1989	4	1.23 [0.81, 1.88]	66% [0.33]
1990-1999	8	1.30 [1.13, 1.50]	24% [0.0003]
2000-2009	8	1.25 [1.14, 1.37]	18% [< 0.00001]
2010 and beyond	7	1.31 [1.18, 1.45]	44% [< 0.00001]
3. Talc Exposure			
3.1. Frequency of Use	7		35% [< 0.00001]
Low	5	1.22 [0.96, 1.54]	54% [0.10]
Medium	2	1.22 [0.98, 1.53]	0% [0.08]
High	7	1.39 [1.22, 1.58]	23% [< 0.00001]
3.2. Duration of Use	6		5% [0.0008]
<10 Years	5	1.22 [1.03, 1.45]	0% [0.02]

Outcome or Subgroup	Studies	Effect Estimate [95% CI)	Heterogeneity I^2 Statistic [p-value]
10 - <20 Years	2	1.42 [1.02, 1.99]	0% [0.04]
20+ Years	2	1.19 [0.71, 1.98]	75% [0.51]
3.3. Method of Use	13		52% [0.001]
Sanitary Napkin	11	1.12 [0.91, 1.39]	50% [0.29]
Diaphragm	10	0.87 [0.72, 1.05]	25% [0.14]
Underwear	2	1.70 [1.27, 2.28]	0% [0.0004]
Male Condom	3	0.99 [0.73, 1.32]	0% [0.92]
4. Tumor Histology			
4.1. Tumor Histology	8		23% [< 0.00001]
Serous	7	1.38 [1.22, 1.56]	0% [< 0.00001]
Mucinous	5	1.05 [0.85, 1.29]	23% [0.41]
Endometrioid	6	1.39 [1.05, 1.82]	2% [0.03]
Clear Cell	1	0.63 [0.15, 2.65]	
5. Tumor Behavior			
5.1. All Grades	4		0% [< 0.00001]
All Invasive	3	1.38 [1.15, 1.65]	0% [0.0004]
All Borderline	4	1.43 [1.08, 1.89]	19% [0.01]
5.2. Serous	5		0% [< 0.00001]
Serous Invasive	5	1.32 [1.13, 1.54]	24% [0.00004]
Serous Borderline	3	1.39 [1.09, 1.78]	0% [0.008]
5.3. Mucinous	3		38% [0.40]
Mucinous Invasive	2	1.34 [0.48, 3.79]	70% [0.58]
Mucinous Borderline	3	1.18 [0.76, 1.82]	34% [0.46]
5.4. Endometrioid	1		N/A
Endometrioid Invasive	1	1.38 [1.06, 1.80]	
5.5. Clear Cell	1		N/A
Clear Cell Invasive	1	1.01 [0.65, 1.57]	
6. Modifiers			
6.1. Menopausal State	2		78% [0.007]
Pre-menopausal	2	1.42 [1.16, 1.75]	0% [0.0008]
Post-Menopausal (HT)	2	2.28 [1.72, 3.01]	0% [< 0.00001]
Post-Menopausal (no HT)	2	1.05 [0.84, 1.32]	25% [0.66]

Outcome or Subgroup	Studies	Effect Estimate [95% CI)	Heterogeneity I^2 Statistic [p-value]
6.2. Pelvic Surgery	7		78% [0.35]
Tubal Ligation	3	0.64 [0.45, 0.92]	19% [0.02]
Hysterectomy	4	0.89 [0.54, 1.46]	61% [0.65]
Combined	4	1.06 [0.78, 1.42]	61% [0.72]

*** NOS:** Newcastle-Ottawa Scale for quality scoring of observational studies

**** Low:** Once daily for 1 – <10 days/month; **Medium:** Once daily for 10 –25 days/month; **High:** Once daily for >25 days/month

3.5. Exposure-Response Assessment

The effect of increasing frequency or duration of perineal use of talc and the risk of ovarian cancer was assessed in the majority of the studies included in this review. Conflicting findings were reported on the nature of the exposure-response relationship: 11 studies concluded that there is no exposure-response, five studies reported a significant positive trend with either frequency or duration of talc use, and two studies concluded that there might be an exposure-response. The remaining twelve studies did not perform or report on trend analyses.

Findings from the seven studies that indicated a potential increased risk of ovarian cancer associated with increasing use of talc are presented in Table 4. The study by Cramer et al. [15] provides the strongest evidence of an exposure-response relationship and could be considered as a key study for exposure-response assessment. The data used in this study were generated from the Nurses' Health Study

originally conducted by Belanger et al. [71], a well-designed high quality cohort study of the factors affecting women's health. The results of this study show an increased risk of ovarian cancer at the three highest exposure categories in this study, with the risk at the lowest exposure level [OR: 1.15 (95% CI: 0.89 to 1.47)] being numerically, although not significantly, elevated. Other studies in Table 4 have provided findings in support of an exposure response based on increasing number of talc applications [20, 30, 34].

In order to permit more direct comparisons of the exposure-response findings from these studies, and whenever the original study data permits, we standardized exposure measurements into talc-years as shown in Figure 3. Data points were selected from studies after excluding potential data points that are lacking precise information on the level of exposure to talc. The mid-point of the exposure categories in the exposure-response studies was used for exposure-response assessment.

Overall, the graphical results shown in this Figure 3 suggest a possible increasing trend in ovarian cancer risk with increasing cumulative exposure to talc; however, there is also a high degree of uncertainty surrounding many of the individual risk estimates. (A formal statistical test for trend was not attempted because of the high degree of heterogeneity among studies noted previously in our meta-analysis discussed in section 3.4.)

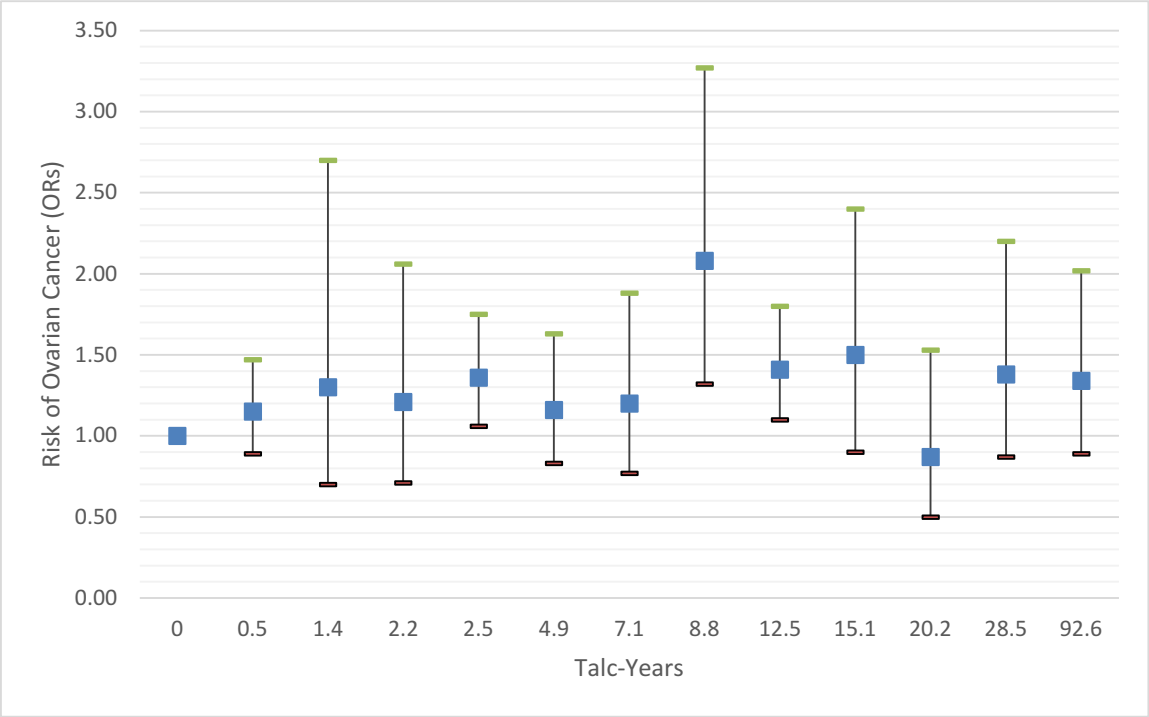

Figure 3: Ovarian cancer risk estimates at increasing levels of exposure to talc, as reported from multiple studies

405 **Table 4: Summary of studies that reported ORs for increasing number of lifetime perineal talc applications**

Study	Stratification	Reported Exposure-Response Strata	aOR*	95% CI
Schildkraut et al. (2016) [30]	Lifetime genital powder	<3,600 applications, any genital use vs (never use)	1.16	[0.83, 1.63]
		>3,600 applications, any genital use vs (never use)	1.67	[1.23, 2.26]
Whittemore et al. (1988) [32]	Overall trend	Overall trend for 30 uses per month	1.3	[0.88, 1.92]
Wu et al. (2009) [34]	By total times of talc use	≤ 5,200 times vs nonuse	1.2	[0.77, 1.88]
		5,201 – 15,600 times vs nonuse	1.38	[0.87, 2.20]
		15,601 – 52,000 times vs nonuse	1.34	[0.89, 2.02]
		> 52,000 times	1.99	[1.34, 2.96]
Mills et al. (2004) [25]	By cumulative use (frequency × duration)	First quartile (lowest exposure)	1.03	[0.59, 1.80]
		Second quartile	1.81	[1.10, 2.97]
		Third quartile	1.74	[1.11, 2.73]
		Fourth quartile (highest exposure)	1.06	[0.62, 1.83]
Rosenblatt et al. (2011) [29]	By lifetime number of applications of perineal powder after bathing	1-1,599 applications	1.21	[0.71, 2.06]
		1,600-4,799 applications	2.08	[1.32, 3.27]
		4,800-9,999 applications	0.87	[0.50, 1.53]
		≥10,000 applications	0.87	[0.48, 1.57]
Cramer et al. (2016) [15]	By total genital applications	≤360 total genital applications	1.15	[0.89, 1.47]
		361-1,800 total genital applications	1.36	[1.06, 1.75]
		1,801-7,200 total genital applications	1.41	[1.10, 1.80]
		>7,200 total genital applications	1.39	[1.11, 1.75]
Harlow et al. (1992) [20]	Total Lifetime Perineal Applications*	< 1,000 applications	1.3	[0.7, 2.7]
		1,000 - 10,000 applications	1.5	[0.9, 2.4]
		>10,000 applications	1.8	[1.0, 3.0]

406 * aOR: adjusted odds ratio

407 ** 10,000 applications are equivalent to daily use for 30 year

4. Discussion

The present analysis of the association between perineal use of talc powder and ovarian cancer risk considered four decades of scientific work exploring the epidemiological associations and non-human studies. The motivation for this review is based on two questions: what do human epidemiology studies of perineal talc exposure reveal about potential ovarian carcinogenicity, and what do in-vitro and in-vivo studies suggest about potential mechanisms of toxicity?

A systematic review of the human epidemiology studies was conducted to address the first question. Thirty observational epidemiologic studies were identified and assessed for quality using the NOS [6]. In parallel with the review of human epidemiological evidence, a (non-systematic) review of evidence exploring in vitro and in vivo toxicology data on talc was conducted to explore how talc might produce biological changes. This latter review provides some insights concerning possible mechanisms of talc toxicity, including oxidative stress, immune system alterations and inflammatory responses. However, it also indicates that talc is not genotoxic. In total, the epidemiology studies suggest that perineal exposure to talc powder is a possible human ovarian carcinogen but there are concerns that the actual exposure experienced by these women over the past 40-50 years is not well understood. As reported by Langesth and colleagues [67], there had been some concern that asbestos-contaminated talc powder that was produced prior to 1976 might have been a confounder; however, the similarity of findings between studies published prior to and after this point suggests asbestos contamination does not explain the positive association between perineal use of talc powder and risk of ovarian cancer [25, 27].

Human observational studies have inherent limitations that could bias the findings. Potentially important sources of bias reported in the included studies include: 1) selection bias due to low response rates from cases and controls or from limiting subjects to English-speaking women of two specific races, and 2) exposure misclassification due to recall bias inherent in case control studies. Other limitations included small sample sizes in some studies, small numbers of subjects in subgroup analyses, lack of information on duration of talc use in many studies that only compared ever vs never users, as well as lack of information on the talc content of the different brands of genital powders used. In two of the three cohort studies, the follow-up period between exposure assessment and end of study could have been inadequate to detect a potential association between talc exposure and ovarian cancer. Houghton et al. [39] reported a mean follow up of 12.4 years, while Gates et al. [36] followed a cohort of women for 24 years. However, Gertig et al. [37] and Gonzalez et al. [38] noted that one of their main limitations is the relatively short follow up periods that may not be adequate to detect a potential association between talc exposure and ovarian cancer. For example, studies of smoking and ovarian cancer suggest that follow-up periods as long as four decades improve recognition of the carcinogenic effects of smoking [72]; longer follow up periods may also improve characterization of the association between talc and ovarian cancer. In this regard, the minimum latency period for radiation-induced ovarian cancer among Hiroshima atomic bomb survivors has been reported to range from 15 to 20 years [73, 74]. Common strengths reported in most studies were the selection of population controls in many of the case control studies and having relatively large sample sizes that allowed a multitude of stratified analyses.

Effect estimates in this meta-analysis were pooled from 24 case control studies and 3 cohort studies, and refer to ever vs never use of perineal talc. Pooling by study design showed a notably higher risk estimate for case-control [OR: 1.32 (95% CI: 1.24 to 1.40), P < 0.00001, I^2= 22%] compared to cohort studies [OR: 1.06 (95% CI: 0.9 to 1.25), P= 0.49, I^2= 17%]. Although the reasons for this are unclear, the difference could potentially be due to issues relating to latency, study power, or exposure misclassification.

Although cohort study designs are efficient for examining diseases with a long latency period, it is essential that the period between talc exposure and the cancer diagnosis be sufficiently long. Gonzalez et al. [38] suggested that the latency period for ovarian cancer is between 15 to 20 years. In the cohort studies included in this review, Houghton et al. [39] reported a mean follow up of 12.4 years while Gates et al. [36] followed a cohort of women for 24 years. Gertig et al. [37] and Gonzalez et al. [38] noted that one of their studies' main limitations was the relatively short follow up periods that may not be adequate to detect a potential association between talc exposure and ovarian cancer.

In addition, cohort studies included may have been underpowered to detect an odds ratio (relative risk) of 1.3 estimated from the case control studies. This was noted by Narod et al. [75], who suggest that cohorts of at least 200,000 women would be needed to reach statistical significance if the true odds ratio is 1.3. The cohort studies included in this review included much smaller cohort sizes, ranging between 41,654 and 78,630 women.

Finally, in cohort studies, talc exposure was assessed at cohort entry and was used as a measure of chronic talc use during follow up. It is possible that women who were not exposed to perineal talc at the time of cohort entry began using talc at a later time, and vice versa, possibly introducing non-differential misclassification of exposure, which could bias the risk estimate towards the null value of unity. Conversely, in the presence of differential exposure misclassification, a bias away from the null hypothesis could accentuate differences between the cohort and case-control studies.

4.1. Exposures and outcomes

All epidemiological studies included in our review evaluated the association between the perineal application of talc and subsequent diagnosis of ovarian cancer. Perineal vs body exposure is an important distinction, as the movement of talc is thought to follow an ascending path from the perineum through the vagina, uterus and fallopian tubes to the ovarian (as well as fallopian tube and peritoneal) epithelium.

Ovarian cancer is a common gynecologic malignancy in developed and developing countries. Risk factors for ovarian cancer include age, infertility, nulligravidity, endometriosis, hereditary ovarian cancer, tobacco and asbestos.

Protective factors for ovarian cancer include oral contraceptives, bilateral tubal ligation, salpingo-oophorectomy, hysterectomy, and breast feeding [76]. It is a difficult cancer to diagnose early, with approximately 60% of the individuals diagnosed after the cancer has metastasized from the pelvic region, where this cancer begins. In the meta-analysis, comparing ovarian cancer risk among women who used talc versus those who

never used talc (using both case-control and cohort designs), we observed an approximate 30% increase in ovarian cancer risk in the group who used talc. The most common type of ovarian cancer seen in the general population, and among the women exposed to talc were of epithelial origin, most common histologic type (accounting for about 95% of all cases in the general population), and of serous morphology, the most common subtype (comprising about 75% in the general population).

The cell-type of origin and morphology of talc induced ovarian cancer is similar to that observed in typical ovarian cancer with approximately 95% derived from epithelium (from fallopian tube fimbriae, ovarian or peritoneal) with serous tumors as the most common subtype. Like most ovarian cancers, those associated with talc exposure are typically diagnosed late in the course of the disease (~60% are diagnosed after the disease has spread outside of the pelvis). This late diagnosis complicates our understanding of the history and origin of the disease.

Demographic factors were analyzed using subgroup analysis where possible, and these were generally consistent with what has been previously observed with respect to ethnicity and risk of ovarian cancer. Additionally, these data also provide support for a mechanism of ovarian cancer induction working via an inflammatory pathway associated with oxidative stress [27, 77, 78].

A small number of studies explored the issue of ethnicity: Asians (1 study), Hispanics (2 studies), and African-Americans and Whites (3 studies each). Among these studies the risk for talc associated ovarian cancer was 1.70 (Hispanics), 1.67 (African Americans), 1.28 (Whites) and 0.04 (Asians). These risk factors compare with the demographics of ovarian cancer in the US population with an overall prevalence of

ovarian cancer of 12.7/100,000 among Whites 13.4/100,00, Hispanics 11.3/100,000, African Americans 9.8/100,000, and Asians 9.8/100,000. The difference in US prevalence and risk of talc induced ovarian cancer among Hispanics and African Americans may provide further evidence concerning exposures or mechanism of action [76].

A variety of factors were assessed with respect to the studies contributing to the meta-analysis, including study quality (NOS) and publication year. In general, the risk of talc associated ovarian cancer was similar among studies with an NOS ≥7 or NOS <7. Year of publication also failed to demonstrate a significant impact on reported talc risk estimates.

4.2. Exposure metrics

Given that the epidemiological studies indicate that talc is a possible human carcinogen, we next evaluated the studies to identify those comparing differences in exposure. The initial assessment exploring frequency of use, utilized a qualitative exposure metric: low, medium and high. Ovarian cancer was observed to increase between the medium and high exposure groups, consistent with an exposure-response relationship. Several studies explored duration of use (years) and risk of ovarian cancer; 20+ years (2 studies),10 (5 studies), 10/20 (2 studies), and observed that the risk was greatest in the 20+ year exposure group, followed by lower risk in the 10/20 year and <10-year exposure groups.

Several studies explored the route of exposure or approach to talc application on ovarian cancer risk, including; hysterectomy, bilateral tubal ligation, diaphragm,

underwear, sanitary napkin, as these can provide insight into differences in exposure of the fallopian tube, ovarian and peritoneal epithelium. Use of a diaphragm, as well as tubal ligation act to interrupt exposure of perineal talc to reproductive tract. In contrast, application to underwear and sanitary napkin exposure will provide broader exposures. As hypothesized, the use of diaphragm and bilateral tubal ligation decreased ovarian cancer risk [22].

4.3. Modifying Factors

Modifiers of the risk of ovarian cancer, either associated with talc exposure, or a spontaneous disease, can provide clues to potential mechanisms of causation. Menopausal status and use of hormones can modify the risk for ovarian cancer. For example, among post-menopausal women receiving hormonal therapy the risk for ovarian cancer is greater than those who are premenopausal and those who are post-menopausal not receiving hormone therapy. It has also been observed that women receiving fertility treatment who do not become pregnant are at greater risk for ovarian cancer [22]. These data suggest that hormonal status (elevated estrogens and/or gonadotropins) plays a role in the mechanism of action of talc associated ovarian cancer.

Subgroup analyses in the meta-analysis indicated that interruption of the pathway from perineum to pelvis (as with bilateral tubal ligation or use of diaphragm) decreased risk for ovarian cancer. This supports the hypothesis that talc acts by local action on the ovary. Given the data developed in non-human studies suggesting an inflammatory response of epithelial cells to talc, and histological observations

corroborating those observations, additional support for an inflammatory pathway leading to ovarian cancer is provided. One study recently explored the use of anti-inflammatory drugs and observed a decreased risk for ovarian cancer, also supporting the importance of an inflammatory pathway with oxidative stress [77].

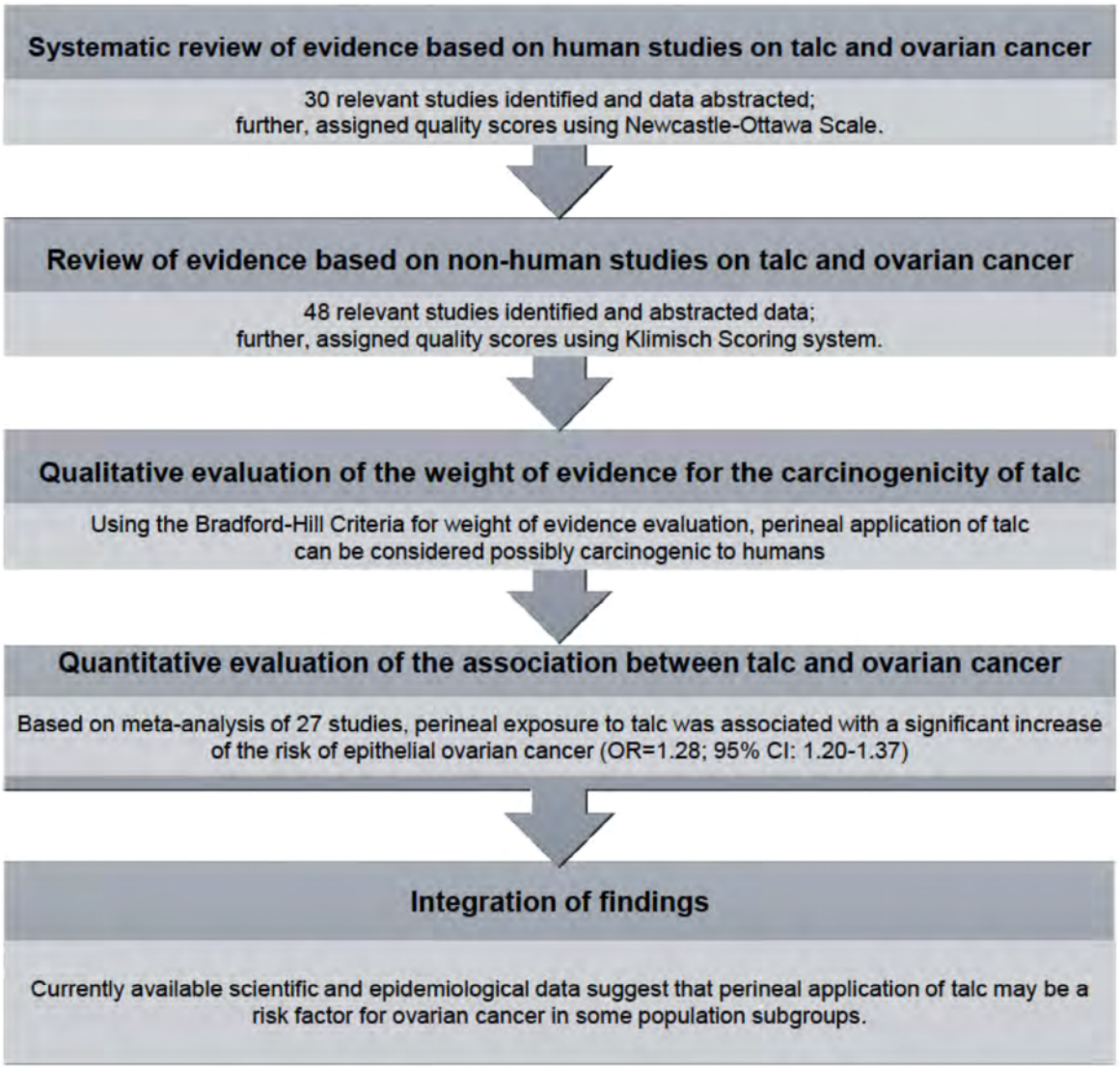

Figure 4: Detailed process flow for assessment of talc carcinogenicity

5. Conclusion

We conducted an extensive search, examination, assessment and analysis of evidence from published human and non-human original as well as all published reviews that considered the association between genital/perineal use of talc powder and risk of ovarian cancer. The steps followed in conducting this review are summarized in Figure 4, along with the key findings at each step. Consistent with previous evaluations the IARC in 2010 [2], and subsequent evaluations by individual investigators [3, 5, 69], the present comprehensive evaluation of all currently available relevant data indicates that perineal exposure to talc powder is a possible cause of ovarian cancer in humans.

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Talc use, variants of the GSTM1, GSTT1, and NAT2 genes, and risk of epithelial ovarian cancer

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Abstract

Epidemiologic evidence suggests a possible association between genital use of talcum powder and risk of epithelial ovarian cancer; however, the biologic basis for this association is not clear. We analyzed interactions between talc use and genes in detoxification pathways (*GSTM1*, *GSTT1* and *NAT2*) to assess whether the talc/ovarian cancer association is modified by variants of genes potentially involved in the response to talc. Our analysis included 1,175 cases and 1,202 controls from a New England-based case-control study and 210 cases and 600 controls from the prospective Nurses' Health Study. We genotyped participants for the *GSTM1* and *GSTT1* gene deletions and three *NAT2* polymorphisms. We used logistic regression to analyze the main effect of talc use, genotype, and gene-talc interactions in each population, and we pooled the estimates using a random effects model. Regular talc use was associated with increased ovarian cancer risk in the combined study population (relative risk=1.36, 95% CI=1.14-1.63; *p*-trend<0.001). Independent of talc, the genes examined were not clearly associated with risk. However, the talc/ovarian cancer association varied by *GSTT1* genotype and combined *GSTM1/GSTT1* genotype. In the pooled analysis, the association with talc was stronger among women with the *GSTT1*-null genotype (*p*-interaction=0.03), particularly in combination with the *GSTM1*-present genotype (*p*-interaction=0.03). There was no clear evidence of an interaction with *GSTM1* alone or *NAT2*. These results suggest that women with certain genetic variants may have a higher risk of ovarian cancer associated with genital talc use. Additional research is needed on these interactions and the underlying biologic mechanisms.

Keywords

Talc; GSTM1; NAT2; ovarian cancer; gene-environment interactions

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INTRODUCTION

Genital use of talcum powder has been extensively investigated as a potential risk factor for ovarian cancer. A meta-analysis of 16 previous studies reported an approximate 30% increase in risk of total epithelial ovarian cancer with regular genital exposure to talc (1), and several studies have suggested a stronger association with the serous or serous invasive histologic subtype (2-6). Although the epidemiologic evidence supports a modest association between genital talc use and ovarian cancer risk, the association remains controversial due to the lack of a clear dose-response with increasing frequency or duration of talc use, the possibility of confounding or other biases, and the uncertain biologic mechanism.

No prior studies have assessed gene-talc interactions in ovarian cancer risk, possibly because little is known about which genes may be involved in the biologic response to talc. However, variants of the *glutathione S-transferase M1* (*GSTM1*) and *N-acetyltransferase 2* (*NAT2*) genes appear to modify the association between exposure to asbestos, a known carcinogen that is chemically similar to talc, and risk of malignant mesothelioma (7-10). Talc and asbestos are found together in nature, and prior to 1976 talcum powder was commonly contaminated with asbestos (9). Although this contamination may have contributed to the risk of ovarian cancer associated with talc use, there is also evidence that talc itself may contribute to carcinogenesis, independent of any contamination with asbestos in the past. Talc can induce granulomas and other inflammatory responses *in vivo* (9), and a recent study found that exposing human ovarian stromal and epithelial cells to talc resulted in increased cell proliferation and neoplastic transformation of cells (11). Talc also appears to increase cellular production of reactive oxygen species (11). Interestingly, serous ovarian cancers morphologically resemble peritoneal malignant mesotheliomas (12), suggesting a possible rationale for the stronger association between talc and risk of serous or serous invasive cancers observed in some studies.

Based on similarities between talc and asbestos and the evidence for gene-asbestos interactions in malignant mesothelioma, we examined whether the association between genital talc exposure and ovarian cancer risk is modified by variants of the *NAT2* and *GSTM1* genes, as well as the related *glutathione S-transferase T1* (*GSTT1*) gene. The *GSTM1* and *GSTT1* genes produce enzymes involved in the metabolism of carcinogens and reactive oxygen species (13). These genes are homozygously deleted in approximately 50% (*GSTM1*) and 20% (*GSTT1*) of Caucasians, resulting in complete loss of enzymatic activity (14,15). The NAT2 enzyme catalyzes the deactivation of xenobiotics via *N*-acetylation, but can also activate certain substrates via *O*-acetylation (16). Individuals with two *NAT2* slow acetylator alleles, approximately 60% of individuals in Caucasian populations, have decreased rates of *N*- and *O*-acetylation (17-20). We hypothesized that the association between talc use and ovarian cancer risk would be stronger among individuals with the *GSTM1* null, *GSTT1* null, and *NAT2* slow acetylator genotypes, due to decreased metabolism of free radicals and other products of the biologic response to talc. We examined these gene-talc interactions, as well as the main effect of talc use and each genotype, in two study populations with a total of 1,385 ovarian cancer cases.

METHODS

New England Case-Control Study

The New England Case-Control Study (NECC) consists of 1,231 epithelial ovarian cancer cases and 1,244 controls from Massachusetts and New Hampshire. Participants were enrolled in the study in two phases, from May 1992 to March 1997 (phase 1; 563 cases and 523 controls) or from July 1998 to July 2003 (phase 2; 668 cases and 721 controls). Participants completed a detailed questionnaire on potential risk factors for ovarian cancer and covariates of interest during an in-person interview with a trained interviewer. To avoid capturing changes related

to disease status, interviewers asked participants about exposures that occurred at least one year prior to the date of diagnosis for cases or the interview date for controls. The institutional review boards of Brigham and Women's Hospital and Dartmouth Medical School approved both phases of the study, and all participants provided written informed consent.

During the two study phases, NECC researchers identified 2,347 incident cases of ovarian cancer through hospital tumor boards and state cancer registries; 1,845 (79%) of these cases were eligible, and 71% of the eligible cases were enrolled in the study. Study investigators identified potential controls using random digit dialing, drivers' license records, and Massachusetts town resident lists. Controls were frequency-matched to cases by age and state of residence. Of the potentially eligible controls contacted by investigators during phase 1, 68% were eligible and agreed to participate. During phase 2, 197 potential controls declined to be contacted by returning a postcard to “opt out” of the study; of the remaining potentially eligible controls who were contacted, 67% were eligible and enrolled in the study. The eligibility criteria and the reasons for non-enrollment of eligible cases are described elsewhere (21).

Over 95% of study participants provided a blood specimen at study enrollment. NECC researchers separated the heparinized blood samples into plasma, red blood cell, and buffy coat (white blood cell) components, extracted DNA from the buffy coat using Qiagen DNA extraction (Qiagen Inc., Valencia, CA), and stored the extracted DNA in freezers at a temperature of -80°C.

Nurses' Health Study

In 1976, 121,701 female registered nurses between the ages of 30 and 55 responded to a mailed questionnaire about known and suspected risk factors for disease, leading to the establishment of the Nurses' Health Study (NHS). Study participants completed follow-up questionnaires every two years, providing information on new diagnoses of disease and updated information on risk factors. Participation in the study has remained high throughout follow-up; between 1976 and 2004 the percentage of follow-up information obtained (questionnaire responses plus deaths) was 95.3%. The corresponding follow-up percentages for women who provided a white blood cell or cheek cell specimen were 98% and 99%, respectively. The Institutional Review Board of Brigham and Women's Hospital, Boston, MA approved both the NHS and this analysis, and all participants provided implied consent by completing and returning the baseline questionnaire.

In 1989 and 1990, 32,826 participants submitted a blood sample for use in genetic and other biomarker analyses. Details of the blood collection are described elsewhere (22). Between 2001 and 2004, 33,040 women without a blood specimen provided a buccal cell specimen. We used a mouthwash protocol to collect the buccal cell samples, based on evidence that this method provides slightly higher DNA yield and quality, compared with collection using a cytobrush (23). We extracted DNA from each specimen within one week of receipt using Qiagen DNA extraction (Qiagen Inc., Valencia, CA), and stored the DNA at -80°C.

NHS nested case-control study

We collected information on new diagnoses of ovarian cancer on each questionnaire, and we also obtained information on deaths due to ovarian cancer through family members, the National Death Index, and the U.S. Postal Service. We confirmed each diagnosis using methods described previously (24). For this analysis, we included all cases with a DNA specimen available from prior to diagnosis (incident cases), as well as cases who submitted a DNA specimen within four years after diagnosis (prevalent cases). We included the prevalent cases in the analysis due to the similarity of characteristics of these cases and the incident cases, and

also because the interval of four years between diagnosis and DNA collection was less than the average survival time of 65.7 months for the incident cases. All cases were diagnosed prior to June 1, 2004 and had no history of a prior cancer, other than non-melanoma skin cancer.

We randomly selected three controls per case from the study participants who gave a buccal cell or blood specimen, who had not had a bilateral oophorectomy prior to the date of diagnosis of the matched case, and who had no history of cancer, other than non-melanoma skin cancer, as of the cycle of diagnosis of the case. We excluded 30 controls from the analysis due to unavailability of genotyping data (n=28) or because the participant was later diagnosed with ovarian cancer and was included in the analysis as a case (n=2). Cases and controls were matched on month and year of birth, DNA type, and menopausal status at diagnosis. For the blood collection, cases and controls were additionally matched on menopausal status and postmenopausal hormone (PMH) use status at blood draw, month/year and time of day of blood draw, and fasting status at blood draw, since these control selections were also used for analyses of plasma hormones and other biomarkers (25).

Exposure assessment

The phase 1 and 2 NECC questionnaires included multiple questions about regular use of talcum, baby or deodorizing powder as an adult. Specific questions asked about type of use (as a dusting powder to the genital area, sanitary napkins, underwear, or non-genital areas), frequency of use, age at first use, number of years used, and brand of powder used. The 1982 NHS questionnaire requested information on whether the participant had ever commonly applied talcum, baby, or deodorizing powder to the perineal area (no, <once/week, 1-6 times/week, or daily) or to sanitary napkins (yes/no). For this analysis, we defined regular genital talc use as application of powder to the genital/perineal region at least once per week. We also created a categorical variable for frequency of talc use, using the categories from the NHS questionnaire.

Genotyping methods

Genotyping was performed at the Dana Farber/Harvard Cancer Center High Throughput Genotyping Core (for the *NAT2* polymorphisms and NHS *GSTM1* and *GSTT1* gene deletions) and the Molecular Epidemiology Research Laboratory at the Harvard School of Public Health (for the NECC *GSTM1* and *GSTT1* gene deletions). All samples were genotyped for three single nucleotide polymorphisms that identify the *NAT2*5*, *NAT2*6*, and *NAT2*7* alleles. These alleles account for over 99% of slow acetylator alleles in Caucasian populations (16,26). The *NAT2* I114T (rs1801280), R197Q (rs1799930), and G286E (rs1799931) polymorphisms were genotyped using the 5' nuclease assay (Taqman) on the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA), in 384-well format. Individuals with two slow acetylator alleles were classified as *NAT2* slow acetylators, while individuals with zero or one slow acetylator allele were classified as rapid acetylators.

The NECC samples were genotyped for the *GSTM1* and *GSTT1* gene deletions using multiplex polymerase chain reaction (PCR), and the PCR products were resolved on a 1.5% agarose gel. The NHS samples were genotyped for the two gene deletions using Taqman realtime PCR in 384-well format. For both the multiplex and real-time PCR assays, individuals were considered to have the *GSTM1* or *GSTT1* null genotype if no PCR product was present for the respective gene; all other individuals were classified as *GSTM1* or *GSTT1* present.

All DNA samples were whole genome amplified prior to genotyping. Laboratory personnel blinded to the case-control status of the samples performed all genotyping, and each plate included blinded replicate samples for quality control purposes. The replicate samples were

100% concordant for all genotypes except the NECC *GSTM1* and *GSTT1* gene deletions, which were 98% and 95% concordant respectively.

Statistical analysis

We used a chi-square test to examine whether the *NAT2* polymorphisms were in Hardy-Weinberg equilibrium in each population, and also to examine the distribution of each genotype by case-control status. We conducted all analyses separately in the NHS and NECC populations using consistent exposure and covariate definitions and, after testing for heterogeneity in the results, pooled the estimates using a random effects model (27). We used conditional (NHS) and unconditional (NECC and NHS) logistic regression to model the multivariable-adjusted odds ratio (as an estimate of the relative risk [RR]) and 95% confidence interval (CI) for the main effect of genital talc use, the main effect of each gene, and each combined gene-talc variable. We tested for a linear trend with increasing frequency of talc use by using a continuous variable weighted by the midpoint of each frequency category, and we calculated the *p*-value for trend using the Wald test. To assess effect modification by genotype, we used unconditional logistic regression to model the association between talc use and ovarian cancer risk within each genotype stratum, and we calculated the *p*-value for interaction using the chi-square test for the difference between the log likelihoods for models with and without interaction terms between regular genital talc use and genotype. In addition to the analyses of total ovarian cancer, we examined associations with the serous invasive histologic subtype, based on evidence from prior studies that risk of this subtype may be more strongly associated with talc use.

We adjusted all analyses for the matching factors, duration of oral contraceptive use, parity, tubal ligation, body mass index (BMI), and duration of PMH use. Women with missing data for the continuous covariates were assigned the median value of the covariate for their study population. In the NHS, where covariate data are available from multiple questionnaire cycles, we used the data from two cycles (two to four years) prior to the cycle of diagnosis for each case and their matched controls, for consistency with the timeframe of the NECC covariate data. We examined additional covariates as potential confounders, including physical activity, smoking history, menopausal status, age at menopause, breastfeeding duration, and family history of ovarian or breast cancer, but did not include them in the final model because they did not substantially change our estimates. We performed all analyses using SAS version 9.1 (SAS Institute Inc., Cary, NC).

RESULTS

Our study population included 1,175 cases and 1,202 frequency-matched controls from the NECC and 210 cases and 600 matched controls from the NHS, for a total of 1,385 ovarian cancer cases and 1,802 controls. Of the NHS cases, 49 were prevalent and 161 were incident with respect to the time of DNA collection. Characteristics of the NHS prevalent and incident cases were generally similar, although a higher percentage of the prevalent cancers were endometrioid (20% vs. 9%) and a lower percentage were invasive (76% vs. 86%). In the NECC, 618 cases had serous histology (53%), 450 were serous invasive (38%), 153 were mucinous (13%), 172 were endometrioid (15%), and 232 had other/undifferentiated histology (20%). In the NHS, 111 cases were serous (53%), 93 were serous invasive (44%), 23 were mucinous (11%), 25 were endometrioid (12%), and 51 had other/poorly differentiated histology (24%).

Over 96% of the NECC participants and 98% of the NHS participants were of self-reported European ancestry. In analyses restricted to these participants, the results were similar to those for the entire study population; we therefore included all participants in our analyses to maximize our sample size. The distributions of ovarian cancer risk factors were similar in the NECC and NHS populations, although on average the NHS participants were older, had higher

parity, and were more likely to have used PMH, in part due to differences in the NECC and NHS age distributions (Table 1). Within each study population the cases and controls differed with respect to the known risk factors for ovarian cancer. In addition, in the NECC the cases had higher mean BMI than the controls, and a larger percentage of the cases reported a history of genital talc use. The NHS prevalent and incident cases had similar BMI, tubal ligation history, duration of PMH use, duration of lactation, and genital talc use history; however, the prevalent cases were, on average, slightly younger (60 vs. 62 years), less likely to be postmenopausal (71% vs. 87%), and had lower parity (2.7 vs. 3.1 children), later age at menarche (13.1 vs. 12.5 years), and a longer mean duration of oral contraceptive use (60 vs. 41 months; results not shown).

In the NECC, women with a history of regular genital talc use were older, had higher mean BMI, were less likely to have ever used oral contraceptives, were more likely to be postmenopausal, and were more likely to have used PMH (Table 2). Among parous women in the NHS, the mean age at first birth was lower for regular talc users. In addition, NHS participants who regularly used talc were less likely to have a history of smoking or tubal ligation. There was no difference in the genotype frequencies by genital talc use history in either study population.

All *p*-values for the tests for heterogeneity comparing the NECC and NHS results were greater than 0.05. Talc use was associated with increased risk of ovarian cancer in both study populations, although the confidence intervals were wide in the NHS due to the limited sample size (Table 3). In the pooled analysis, the relative risk for the association with regular genital talc use was 1.36 (95% CI=1.14-1.63) for total ovarian cancer and 1.60 (95% CI=1.26-2.02) for the serous invasive subtype. In addition, there were highly significant trends between increasing frequency of talc use and risk of both total and serous invasive ovarian cancer in the NECC (*p*-trend=0.002 for total and <0.001 for serous invasive ovarian cancer) and pooled analyses (*p*-trend<0.001 for both total and serous invasive ovarian cancer). Regular genital talc use was not significantly associated with risk of the endometrioid (RR=1.41, 95% CI=0.97-2.05) or mucinous (RR=1.28, 95% CI=0.85-1.92) histologic subtypes in the pooled analysis. In the NECC, use of talcum powder on non-genital body areas was unassociated with ovarian cancer risk (multivariable-adjusted RR, also adjusted for genital talc use=0.91, 95% CI=0.73-1.12).

Among the controls in each population, the genotype frequencies for the *NAT2* polymorphisms were in Hardy-Weinberg equilibrium and the distributions of the *GSTM1* null, *GSTT1* null, and *NAT2* slow acetylator genotypes were consistent with previous reports of Caucasian populations (19,28,29). Comparing the prevalent and incident cases in the NHS, a nonsignificantly higher percentage of the prevalent cases were *NAT2* slow acetylators (67% vs. 56%), but the *GSTM1* and *GSTT1* genotype distributions did not differ for the prevalent and incident cases (results not shown).

None of the genotypes examined were associated with ovarian cancer risk in the NECC or pooled analyses (Table 4). In the NHS, individuals with the *NAT2* slow acetylator genotype had a significant 35% decrease in ovarian cancer risk (RR=0.65, 95% CI=0.45-0.95). The combined *GSTM1* null/*NAT2* slow acetylator and *GSTT1* null/*NAT2* slow acetylator genotypes were also inversely associated with risk in the NHS (RR=0.57, 95% CI=0.33-0.98 and RR=0.51, 95% CI=0.26-0.99, respectively), when compared with the *GSTM1* or *GSTT1* present, *NAT2* rapid acetylator genotype. However, these associations were no longer statistically significant when pooled with the NECC estimates.

In analyses stratified by genotype, the association between regular genital talc use and risk of total ovarian cancer was stronger among women with the *GSTT1* null and combined *GSTM1*

present/*GSTT1* null genotypes (Table 5). In the pooled analysis, the relative risk for the association with regular genital talc use was 2.1 (95% CI=1.4-3.2) for women with the *GSTT1* null genotype (*p*-interaction=0.03) and 2.8 (95% CI=1.6-5.0) for women with the *GSTM1* present/*GSTT1* null genotype (*p*-interaction=0.03). The association with the serous invasive subtype was also stronger within these genotype strata, although the *p*-values for interaction were not statistically significant. The pooled relative risk was 2.4 (95% CI=1.4-4.0) for the *GSTT1* null stratum and 4.8 (95% CI=2.1-11) for the combined *GSTM1* present/ *GSTT1* null stratum. The results were consistent in both study populations (results not shown), although the *p*-values for interaction were statistically significant only in the pooled analysis. There was also evidence of a stronger association between regular talc use and risk of serous invasive cancer among women with the *GSTM1* present genotype, but this interaction was not statistically significant.

We additionally analyzed the association between combined gene-talc variables, compared to a common referent group (wild-type genotype and no talc use), and risk of total and serous invasive ovarian cancer. The results of these analyses were similar to the stratified results presented in table 5, and are therefore included only as a supplementary table. We also examined interactions between regular genital talc use and combined *GSTM1/NAT2* and *GSTT1/NAT2* genotype (results not shown). The *GSTT1* null/*NAT2* slow acetylator genotype seemed to increase the risk of total and serous invasive ovarian cancer associated with talc use. However, these analyses were based on small numbers, especially for certain combinations of the genotype and talc variables, and none of the *p*-values for interaction were significant.

In analyses restricted to the NHS incident cases or the NHS cases and controls with a blood specimen, the results were similar to those for the total NHS study population (results not shown).

DISCUSSION

These results provide additional support for a main effect of genital talc exposure on risk of epithelial ovarian cancer. The presence of a significant trend between frequency of talc use and risk of total and serous invasive ovarian cancer in the NECC and pooled analyses further strengthens the evidence for an association, as most previous studies have not observed a dose-response with increasing frequency or duration of talc use (1,5). The results of our gene-environment analyses suggest that genes in detoxification pathways may be involved in the biologic response to talc, and that the association between genital talc use and risk of ovarian cancer may vary by genotype. In particular, women with the *GSTT1* null genotype and the combined *GSTM1* present/*GSTT1* null genotype had a stronger association between talc use and ovarian cancer risk. The evidence for these interactions was consistent in two independent study populations, and the *p*-values for interaction were statistically significant in a pooled analysis of the two populations. However, the direction of the interaction with combined *GSTM1/GSTT1* genotype was unexpected based on the known function of these genes.

Although prior analyses of the talc/ovarian cancer association in the NHS and the NECC have been published, our study includes an additional 612 NECC cases and 679 NECC controls and eight additional years of follow-up in the NHS (3,4). In the previous analysis of the NECC, Cramer et al. observed a significant positive association between talc use and risk of both total and serous invasive ovarian cancer. In addition, there was a significant trend with lifetime number of talc applications, after excluding applications during non-ovulatory intervals (*p*-trend=0.02), but no trend with duration or frequency of talc use (3). In the only prospective study of this association, Gertig et al. reported a significant association between talc use and risk of the serous invasive subtype in the NHS, but no association with risk of total ovarian cancer (4). Our findings are consistent with the previous reports for these study populations,

although our analysis differs from the prior studies in that we defined our primary exposure variable as genital use of talc at least once per week, based on the assumption that habitual talc use is more likely to be recalled accurately and more likely to be associated with ovarian cancer risk. Our findings are also consistent with meta-analyses of this association (1,30).

The controversy regarding the existence of an association between talc and ovarian cancer has stemmed in part from the lack of a clear mechanism for the association. Although talc and asbestos are chemically similar, their biologic effects may differ, since talc does not appear to be a lung carcinogen (31). In addition, it is unclear whether talc applied to the perineum can reach the ovaries, although some studies have demonstrated that inert particles can travel through the female genital tract to the fallopian tubes and ovaries (32,33), and others have found talc particles in ovarian tissue (34-37). Recent studies have suggested additional potential mechanisms for an association between talc and ovarian cancer. Talc particles can induce an inflammatory response *in vivo*, which may be important in ovarian cancer risk (38). Normal ovarian cells treated with talc are more likely to undergo cell proliferation and neoplastic transformation, and cellular generation of reactive oxygen species increases with increasing exposure to talc (11). Recent studies by Cramer and colleagues also support the possibility of an immune-mediated mechanism for an association between talc and ovarian cancer and suggest that exposure of the lower genital tract to talc may be sufficient to cause changes, such as production of heat shock proteins, accumulation of talc in pelvic lymph nodes, or decreased levels of anti-MUC1 antibodies, that could increase ovarian cancer risk (39-41).

Although no prior studies have examined gene-talc interactions, the indication of a possible immune-related mechanism between talc and ovarian carcinogenesis and the evidence for gene-asbestos interactions suggest that genes involved in detoxification and inflammatory pathways could be important in the response to talc. Previous studies have indicated that *NAT2* and *GSTM1* genotype may modify the association between asbestos exposure and risk of malignant mesothelioma; however, not all studies have been consistent (7,8,42,43), and for *NAT2* the direction of the interaction differed in studies conducted in Finnish and Italian populations (7,8,42,44). This suggests that interactions with these genes may be complex and might depend on additional factors, such as the presence of other gene variants, the type of asbestos, or the level of asbestos exposure (8).

The *GSTM1* and *GSTT1* genes produce enzymes that metabolize products of oxidative stress and catalyze the detoxification of carcinogens and other xenobiotics (45). The *GSTM1* deletion and to a lesser extent the *GSTT1* deletion may increase the risk of certain cancers; however, our study and previous analyses do not support a direct association between the *GSTM1* or *GSTT1* gene deletion and risk of ovarian cancer (13,17,28). While there is some overlap in GST substrate specificity, there are also differences in the substrates metabolized by the GSTM1 and GSTT1 enzymes, which could help to explain the opposite direction of the interactions we observed between talc use and *GSTM1* and *GSTT1* genotype (13,17,45). In studies of pleural malignant mesothelioma, the *GSTM1* null genotype was associated with increased risk (7,8,42,43) while the *GSTT1* null genotype was unassociated with risk of malignant mesothelioma (8,42,43) but was associated with a significant decrease in risk of asbestosis in one study (46), providing support that some functions of the GSTM1 and GSTT1 enzymes may differ. The direction of the associations between the *GSTM1* and *GSTT1* deletions and risk of asbestos-related disease was opposite to the direction of the interactions with talc observed in our study; this could potentially be due to differences in the chemical structures of talc and asbestos or differences in the by-products produced during the biologic response to talc and asbestos. The NAT2 enzyme catalyzes the transfer of an acetyl group to its substrates, including carcinogens such as heterocyclic and aromatic amines, which can result in either activation or deactivation of these substances (17,20). Approximately 60% of Caucasians have two *NAT2* slow acetylator alleles and consequently have decreased rates of acetylation, which

can either increase or decrease the risk of certain cancers depending on the substrate and the cancer site (17,20). To our knowledge, no previous studies have examined the association between the *NAT2* slow acetylator genotype and ovarian cancer risk. We did not observe strong evidence of a main effect of *NAT2* genotype or an interaction between *NAT2* genotype and talc exposure.

The novelty of this analysis and the assessment of gene-talc interactions in two independent study populations, one with a large number of cases and the other with prospective data on talc use and ovarian cancer incidence, are strengths of this study. However, although the pooled analysis included a large number of cases and controls, our power was still insufficient to detect interactions with certain combinations of genes and for specific histologic subtypes. In addition, while both study populations had extensive covariate data, the use of common exposure and covariate definitions resulted in the loss of some detail, particularly for the NECC. Information on talc use was only collected in 1982 in the NHS, so it is possible that some participants were misclassified with respect to their talc use history. However, the number of participants who began using talc after 1982, when the participants were between 36 and 61 years of age, is most likely small. Although we do not have data on age at initiation of talc use in the NHS, in the NECC approximately 95% of controls with a history of regular genital talc use reported first using talc before age 35. Recall or selection bias may have affected the results of the NECC analyses, due to the retrospective study design. However, the consistency of the NECC and NHS results suggests that biases related to study design were not a major problem, since, with the exception of the DNA for a subset of the cases, the NHS data were collected prospectively. In addition, the exposure definition of genital talc use at least once per week may have decreased the influence of recall bias in this analysis, since habitual talc use is likely to be recalled more accurately than sporadic use.

In summary, our findings suggest that variants of the *GSTM1* and *GSTT1* genes may modify the association between genital talc use and risk of total and serous invasive ovarian cancer. However, additional research is needed to confirm these findings and to explore potential mechanisms for these interactions, particularly for the stronger talc/ovarian cancer association among women with the *GSTM1* present/*GSTT1* null genotype. If confirmed, these findings would strengthen the evidence for the carcinogenicity of talc to the ovarian epithelium.

Supplementary Table

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of ovarian cancer cases and controls in the New England Case-Control Study (NECC) and the Nurses' Health Study (NHS)

Characteristic	NECC			NHS[//]		
	Cases	Controls	*P*[**]	Cases	Controls	*P*[**]
N	1175	1202		210	600	
Mean value (standard deviation)						
Age in years[*]	51 (13)	51 (13)	0.37	62 (8)	62 (8)	0.93
Parity among parous women	2.5 (1.3)	2.8 (1.5)	<0.001	3.0 (1.3)	3.3 (1.5)	0.007
Duration oral contraceptive use (months)[†]	52 (54)	61 (55)	0.006	46 (42)	53 (49)	0.22
Body mass index (kg/m^2)	26.3 (6.3)	25.7 (5.5)	0.02	25.7 (5.0)	25.7 (4.5)	0.94
Duration PMH use (months)[†]	78 (86)	74 (71)	0.64	96 (84)	85 (68)	0.18
Duration of lactation (months)[‡]	3.4 (8.6)	5.9 (12.2)	<0.001	2.9 (2.3)	3.6 (2.5)	0.002
Percent of study population						
Parous	68	81	<0.001	89	93	0.05
Ever user of oral contraceptives	48	60	<0.001	42	45	0.57
History of tubal ligation	14	18	0.007	14	21	0.02
Ever user of PMH	17	20	0.14	71	63	0.02
Family history of ovarian cancer	5.1	2.8	0.004	9.1	3.7	0.002
Any history of genital talc use	29	24	0.003	40	39	0.79
Regular genital talc use (>=once/week)	27	20	<0.001	29	24	0.15
Daily genital talc use	16	12	0.006	18	13	0.08
Genotype frequencies, %						
GSTM1 null	51	53	0.42	48	52	0.36
GSTT1 null	21	22	0.85	19	21	0.45
NAT2 slow acetylator[§]	63	64	0.74	59	67	0.05

[*] Cases and controls in each study population were matched (NHS) or frequency-matched (NECC) on age

[†] Duration of oral contraceptive use and postmenopausal hormone (PMH) use among ever users

[‡] Total duration among parous women

[§] *NAT2* acetylation genotype based on analysis of three single nucleotide polymorphisms, I114T, R197Q, and G286E

[//] In the NHS, duration of lactation was collected in 1986, family history of ovarian cancer was first collected in 1992, and history of genital talc use was collected in 1982; for variables collected on multiple questionnaires, the value from two cycles (two to four years) prior to the date of diagnosis for each case was used for the case and their matched controls

[**] *P*-values calculated using proc ttest (continuous variables) or a chi-square test (binary variables)

Table 2

Characteristics of participants in the New England Case-Control Study (NECC) and the Nurses' Health Study (NHS) by history of regular genital talc use (at least once per week)

Characteristic	NECC			NHS[§]		
	No Regular Talc Use	Regular Talc Use	*P*[‖]	No Regular Talc Use	Regular Talc Use	*P*[‖]
Mean value (standard deviation)						
Age in years	50 (13)	53 (12)	<0.001	61 (8)	62 (8)	0.64
Parity among parous women	2.7 (1.4)	2.7 (1.4)	0.64	3 2 (1.4)	3.3 (1.5)	0.40
Age at first birth among parous women	25.0 (5.1)	24.6 (4.9)	0.22	25.0 (3.5)	24.4 (3.0)	0.03
Duration oral contraceptive use (months)[*]	58 (55)	54 (54)	0.24	53 (49)	43 (42)	0.08
Body mass index (kg/m^2)	25.7 (5.7)	27.0 (6.4)	<0.001	25.6 (4.6)	26.2 (4.8)	0.13
Duration PMH use (months)[*]	75 (74)	78 (86)	0.68	90 (74)	83 (70)	0.38
Duration of lactation (months)[†]	4.8 (10.8)	4 3 (10.3)	0.38	3 5 (2.5)	3.2 (2.4)	0.20
Physical activity (hours/week)	2.8 (5.0)	2.4 (3.8)	0.06	3.0 (2.3)	3.1 (2.4)	0.61
Percent of study population						
Parous	74	75	0.75	93	92	0.81
Ever user of oral contraceptives	55	50	0.03	44	44	0.96
History of tubal ligation	16	16	0.97	22	13	0.008
Postmenopause	45	54	<0.001	81	81	0.86
Ever user of PMH	17	26	<0.001	66	64	0.73
Ever smoker	53	55	0.35	57	47	0.02
Family history of ovarian cancer	3.9	4 1	0.82	4.5	6.4	0.31
Genotype frequencies, %						
GSTM1 null	52	52	0.72	51	49	0.66
GSTT1 null	21	22	0.59	22	17	0.15
NAT2 slow acetylator[‡]	63	66	0.23	65	63	0.58

[*] Duration of oral contraceptive use and postmenopausal hormone (PMH) use among ever users

[†] Total duration among parous women

[‡] *NAT2* acetylation genotype based on analysis of three single nucleotide polymorphisms, I114T, R197Q, and G286E

[§] In the NHS, duration of lactation was collected in 1986, family history of ovarian cancer was first collected in 1992, and history of genital talc use was collected in 1982; for variables collected on multiple questionnaires, the value from two cycles (two to four years) prior to the date of diagnosis for each case was used for the case and their matched controls

[‖] *P*-values calculated using proc ttest (continuous variables) or a chi-square test (binary variables)

Table 3

Relative risks (RRs) and 95% confidence intervals (CIs) for the association between genital talc use and ovarian cancer risk in the New England Case-Control Study (NECC) and the Nurses' Health Study (NHS)

	NECC[†]			NHS[†]			Pooled[‡]
	Cases (%)	Ctrls (%)	RR (95% CI)	Cases (%)	Ctrls (%)	RR (95% CI)	RR (95% CI)
Total epithelial ovarian cancer:							
N[*]	1175	1202		210	600		
Regular genital talc use (>=once/week)							
No	859 (73.2)	957 (79.7)	1.00 (ref.)	138 (70.8)	414 (76.0)	1.00 (ref.)	1.00 (ref.)
Yes	314 (26.8)	244 (20.3)	1.40 (1.15, 1.70)	57 (29.2)	131 (24.0)	1.24 (0.83, 1.83)	1.36 (1.14, 1.63)
Frequency of genital talc use							
Never	832 (70.9)	916 (76.3)	1.00 (ref.)	120 (61 5)	352 (64.6)	1.00 (ref.)	1.00 (ref.)
<once/week	27 (2.3)	41 (3.4)	0.72 (0.43, 1.19)	18 (9.2)	62 (11.4)	0.98 (0.54, 1.79)	0.82 (0.55, 1.20)
1-6 times/week	123 (10.5)	96 (8.0)	1.33 (1.00, 1.79)	22 (11.3)	61 (11.2)	1.01 (0.57, 1.79)	1.26 (0.97, 1.63)
Daily	191 (16.3)	148 (12.3)	1.41 (1.10, 1.79)	35 (18.0)	70 (12.8)	1.44 (0.88, 2.37)	1.41 (1.14, 1.76)
P-trend[§]			0.002			0.18	<0.001
Serous invasive ovarian cancer:							
N[*]	450	1202		93	263		
Regular genital talc use (>=once/week)							
No	310 (69.0)	957 (79.7)	1.00 (ref.)	60 (68.2)	177 (73.8)	1.00 (ref.)	1.00 (ref.)
Yes	139 (31.0)	244 (20.3)	1.62 (1.26, 2.09)	28 (31.8)	63 (26.3)	1.48 (0.82, 2.68)	1.60 (1.26, 2.02)
Frequency of genital talc use							
Never	299 (66.6)	916 (76.3)	1.00 (ref.)	54 (61.4)	151 (62.9)	1.00 (ref.)	1.00 (ref.)
<once/week	11 (2.4)	41 (3.4)	0.65 (0.32, 1.33)	6 (6.8)	26 (10.8)	0.79 (0.29, 2.11)	0.70 (0.39, 1.24)
1-6 times/week	56 (12 5)	96 (8.0)	1.56 (1.08, 2.26)	12 (13.6)	25 (10.4)	1.64 (0.71, 3.79)	1.58 (1.12, 2.21)
Daily	83 (18 5)	148 (12.3)	1.61 (1.18, 2.20)	16 (18.2)	38 (15.8)	1.34 (0.65, 2.76)	1.56 (1.17, 2.08)
P-trend[§]			<0.001			0.29	<0.001

[*] Frequencies do not add up to total N due to missing data on talc use

[†] Unconditional (NECC) and conditional (NHS) logistic regression adjusted for age, study center (NECC only), duration of oral contraceptive use (months), parity (continuous), tubal ligation, body mass index (kg/m2, continuous), and duration of postmenopausal hormone use (months)

[‡] *P*-values for tests for heterogeneity comparing the NECC and NHS results were all >0.38

[§] Weighted by the midpoint of each category of genital talc use frequency and calculated using the Wald test

Table 4

Relative risks (RRs) and 95% confidence intervals (CIs) for the association between *GSTM1*, *GSTT1*, and *NAT2* genotype and epithelial ovarian cancer risk in the New England Case-Control Study (NECC) and the Nurses' Health Study (NHS)

	NECC†			NHS†			Pooled‡
	Cases (%)	Ctrls (%)	RR (95% CI)	Cases (%)	Ctrls (%)	RR (95% CI)	RR (95% CI)
N*	1175	1202		210	600		
GSTM1 genotype							
Present	573 (49.1)	567 (47.4)	1.00 (ref.)	102 (52.3)	268 (48.5)	1.00 (ref.)	1.00 (ref.)
Null	594 (50.9)	628 (52.6)	0.93 (0.79, 1 10)	93 (47.7)	285 (51.5)	0.83 (0.58, 1.17)	0.91 (0.78, 1.06)
GSTT1 genotype							
Present	919 (78.8)	938 (78.5)	1.00 (ref.)	157 (81.3)	439 (78.8)	1.00 (ref.)	1.00 (ref.)
Null	247 (21.2)	257 (21.5)	0.98 (0.80, 1 21)	36 (18.7)	118 (21.2)	0.87 (0.57, 1.33)	0.96 (0.80, 1.16)
NAT2 genotype							
Rapid/intermediate acetylator	387 (36.8)	405 (36.1)	1.00 (ref.)	77 (41.0)	182 (33.0)	1.00 (ref.)	1.00 (ref.)
Slow acetylator	665 (63.2)	717 (63.9)	0.97 (0.81, 1 15)	111 (59.0)	369 (67.0)	0.65 (0.45, 0.95)	0.82 (0.57, 1.20)
Combined *GSTM1/GSTT*1 genotype							
Both present	445 (38.2)	430 (36.0)	1.00 (ref.)	81 (44.3)	206 (39.2)	1.00 (ref.)	1.00 (ref.)
M1 null, *T1* present	474 (40.7)	508 (42.5)	0.91 (0.76, 1 10)	68 (37.2)	208 (39.5)	0.82 (0.54, 1.22)	0.89 (0.75, 1.06)
M1 present, *T1* null	128 (11.0)	137 (11.5)	0.94 (0.71, 1 24)	17 (9.3)	49 (9 3)	0 98 (0.52, 1.84)	0.94 (0.73, 1.22)
Both null	119 (10.2)	120 (10.0)	0.94 (0.70, 1 26)	17 (9.3)	63 (12.0)	0.65 (0.34, 1.24)	0.88 (0.67, 1.15)
Combined *GSTM1/NAT2* genotype							
GSTM1 present, *NAT2* rapid	195 (18.6)	188 (16.9)	1.00 (ref.)	37 (21.0)	85 (16.4)	1.00 (ref.)	1.00 (ref.)
GSTM1 null, *NAT2* rapid	189 (18.1)	214 (19.2)	0.82 (0.61, 1.09)	35 (19.9)	91 (17.5)	0 96 (0.53, 1.74)	0.84 (0.65, 1.09)
GSTM1 present, *NAT2* slow	315 (30.1)	343 (30.7)	0.86 (0.66, 1 11)	56 (31.8)	161 (31.0)	0.87 (0.51, 1.50)	0.86 (0.68, 1.09)
GSTM1 null, *NAT2* slow	347 (33.2)	371 (33.2)	0.88 (0.68, 1 14)	48 (27.3)	183 (35.2)	0 57 (0.33, 0.98)	0.76 (0.50, 1.14)
Combined *GSTT1/NAT2* genotype							
GSTT1 present, *NAT2* rapid	296 (28.3)	312 (28.0)	1.00 (ref.)	52 (29.7)	144 (27.5)	1.00 (ref.)	1.00 (ref.)
GSTT1 null, *NAT2* rapid	88 (8.4)	90 (8.1)	1.03 (0.73, 1.45)	17 (9.7)	30 (5.7)	1 55 (0.76, 3.16)	1.11 (0.81, 1.52)
GSTT1 present, *NAT2* slow	519 (49.7)	562 (50.4)	0.97 (0.79, 1 19)	90 (51.4)	270 (51.6)	0.87 (0.57, 1.33)	0.95 (0.79, 1.14)
GSTT1 null, *NAT2* slow	142 (13.6)	152 (13.6)	0.98 (0.74, 1 31)	16 (9.1)	79 (15.1)	0 51 (0.26, 0.99)	0.76 (0.40, 1.43)

*Frequencies do not add up to total N due to missing genotype data

†Unconditional (NECC) and conditional (NHS) logistic regression adjusted for age, study center (NECC only), duration of oral contraceptive use (months), parity (continuous), tubal ligation, body mass index (kg/m2, continuous), and duration of postmenopausal hormone use (months)

‡*P*-values for tests for heterogeneity comparing the NECC and NHS results were all >0.06

Table 5

Pooled relative risks (RRs) and 95% confidence intervals (CIs) for the association between regular talc use and ovarian cancer risk, stratified by genotype, in the New England Case-Control Study (NECC) and the Nurses' Health Study (NHS)[*,†]

	All cancers		Serous invasive cancers		Cases and controls in pooled analysis					
					All cases		Serous inv.		Controls	
	Regular talc use		Regular talc use		Regular talc		Regular talc		Regular talc	
	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
Gene/stratum										
GSTM1 genotype										
Present (+)	1.0 (ref.)	1.6 (1.2, 2.0)	1.0 (ref.)	2.0 (1.4, 2.8)	480	189	173	90	646	165
Null (-)	1.0 (ref.)	1.3 (1.0, 1.6)	1.0 (ref.)	1.4 (1.0, 1.9)	498	179	190	76	690	198
P-interaction[§]	0.13		0.08							
GSTT1 genotype										
Present (+)	1.0 (ref.)	1.2 (1.0, 1.5)	1.0 (ref.)	1.5 (1.2, 2.0)	785	278	288	129	1035	301
Null (-)	1.0 (ref.)	2.1 (1.4, 3.2)	1.0 (ref.)	2.4 (1.4, 4.0)	194	87	71	38	300	67
P-interaction[§]	0.03		0.18							
NAT2 genotype										
Rapid/intermediate acetylator	1.0 (ref.)	1.5 (1.1, 2.0)	1.0 (ref.)	1.9 (1.2, 2.8)	330	128	123	57	459	113
Slow acetylator	1.0 (ref.)	1.4 (1.1, 1.8)	1.0 (ref.)	1.6 (1.2, 2.1)	552	217	204	96	819	233
P-interaction[§]	0.60		0.58							
GSTM1/GSTT1 genotype[‡]										
GSTM1+, *GSTT1* +	1.0 (ref.)	1.4 (1.0, 1.8)	1.0 (ref.)	1.7 (1.2, 2.5)	378	142	136	68	479	140
GSTM1-, *GSTT1*+	1.0 (ref.)	1.2 (0.9, 1.5)	1.0 (ref.)	1.4 (0.9, 1.9)	400	135	151	60	541	154
GSTM1+, *GSTT1*-	1.0 (ref.)	2.8 (1.6, 5.0)	1.0 (ref.)	4.8 (2.1, 11)	98	47	34	22	158	24
GSTM1-, *GSTT1*-	1.0 (ref.)	1.6 (0.9, 2.9)	1.0 (ref.)	1.4 (0.6, 3.1)	94	40	36	16	138	41
P-interaction[§]	0.03		0.09							

[*] NECC: unconditional logistic regression adjusted for age, study center, duration of oral contraceptive use (months), parity (continuous), tubal ligation, body mass index (kg/m2, continuous), and duration of postmenopausal hormone use (months); NHS: unconditional logistic regression adjusted for age in months, menopausal status at diagnosis (post, pre/dubious), DNA source, duration of oral contraceptive use (months), parity (continuous), tubal ligation, body mass index (kg/m2, continuous), and duration of postmenopausal hormone use (months)

[†] *P*-values for tests for heterogeneity comparing the NECC and NHS results were all >0.36

[‡] NHS analysis adjusted for age, menopausal status at diagnosis, and DNA source only, to improve stability of estimates

[§] *P*-values for interaction based on likelihood ratio test comparing unconditional logistic regression models with and without gene-talc interaction terms

Exhibit 61

Review

Inflammation and cancer: back to Virchow?

Fran Balkwill, Alberto Mantovani

The response of the body to a cancer is not a unique mechanism but has many parallels with inflammation and wound healing. This article reviews the links between cancer and inflammation and discusses the implications of these links for cancer prevention and treatment. We suggest that the inflammatory cells and cytokines found in tumours are more likely to contribute to tumour growth, progression, and immunosuppression than they are to mount an effective host anti-tumour response. Moreover cancer susceptibility and severity may be associated with functional polymorphisms of inflammatory cytokine genes, and deletion or inhibition of inflammatory cytokines inhibits development of experimental cancer. If genetic damage is the "match that lights the fire" of cancer, some types of inflammation may provide the "fuel that feeds the flames". Over the past ten years information about the cytokine and chemokine network has led to development of a range of cytokine/chemokine antagonists targeted at inflammatory and allergic diseases. The first of these to enter the clinic, tumour necrosis factor antagonists, have shown encouraging efficacy. In this article we have provided a rationale for the use of cytokine and chemokine blockade, and further investigation of non-steroidal anti-inflammatory drugs, in the chemoprevention and treatment of malignant diseases.

It was in 1863 that Rudolf Virchow noted leucocytes in neoplastic tissues and made a connection between inflammation and cancer. He suggested that the "lymphoreticular infiltrate" reflected the origin of cancer at sites of chronic inflammation. Over the past ten years our understanding of the inflammatory microenvironment of malignant tissues has supported Virchow's hypothesis, and the links between cancer and inflammation are starting to have implications for prevention and treatment.

Panel 1 lists some cancers where the inflammatory process is a cofactor in carcinogenesis. About 15% of the global cancer burden is attributable to infectious agents,[1] and inflammation is a major component of these chronic infections. Moreover, increased risk of malignancy is associated with the chronic inflammation caused by chemical and physical agents,[2] and autoimmune and inflammatory reactions of uncertain aetiology.[3]

Inflammatory cells in tumour microenvironment

The inflammatory microenvironment of tumours is characterised by the presence of host leucocytes both in the supporting stroma and in tumour areas.[4] Tumour-infiltrating lymphocytes may contribute to cancer growth and spread, and to the immunosuppression associated with malignant disease.

Macrophages

Tumour-associated macrophages (TAM) are a major component of the infiltrate of most, if not all, tumours.[5] TAM derive from circulating monocytic precursors, and are directed into the tumour by chemoattractant cytokines called chemokines. Many tumour cells also produce cytokines called colony-stimulating factors that prolong survival of TAM. When appropriately activated, TAM can kill tumour cells or elicit tissue destructive reactions centred on the vascular endothelium. However, TAM also produce growth and angiogenic factors as well as protease enzymes which degrade the extracellular matrix. Hence, TAM can stimulate tumour-cell proliferation, promote angiogenesis, and favour invasion and metastasis.[6] Direct evidence for the importance of protease production by TAM, neutrophils, and mast cells during experimental carcinogenesis has recently been reported.[7] This dual potential of TAM is expressed in the "macrophage balance" hypothesis.[5]

Dendritic cells

Dendritic cells have a crucial role in both the activation of antigen-specific immunity and the maintenance of tolerance, providing a link between innate and adaptive immunity. Tumour-associated dendritic cells (TADC) usually have an immature phenotype with defective ability to stimulate T cells.[8] In breast cancer, immature TADC are interspersed in the tumour mass, whereas mature dendritic cells are confined to the peritumoral area.[8] In papillary thyroid carcinoma TADC are also immature but they tend to localise at the invasive edge of the tumour.[8] This distribution of TADC is clearly different from that of TAM, which are evenly scattered in tumour tissue. The immaturity of TADC may reflect lack of effective

Panel 1: **Some associations between inflammation and cancer risk**

Malignancy	Inflammatory stimulus/condition
Bladder	Schistosomiasis
Cervical	Papillomavirus
Ovarian	Pelvic inflammatory disease/talc/tissue remodelling
Gastric	*H pylori* induced gastritis
MALT lymphoma	*H pylori*
Oesophageal	Barrett's metaplasia
Colorectal	Inflammatory bowel disease
Hepatocellular	Hepatitis virus (B and C)
Bronchial	Silica, asbestos, cigarette smoke
Mesothelioma	Asbestos
Kaposi's sarcoma	Human herpesvirus type 8

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maturation signals, prompt migration of mature cells to lymph nodes, or the presence of maturation inhibitors. TADC are likely to be poor inducers of effective responses to tumour antigens.

Lymphocytes

Natural killer cells are rare in the tumour microenvironment.[4] The predominant T-cell population has a "memory" phenotype. The cytokine repertoire of these tumour-infiltrating T cells (TIL) has not been studied systematically but in some tumours (eg, Kaposi's sarcoma, Hodgkin's disease, bronchial carcinoma, and cervical carcinoma) they produce mainly interleukins (IL) 4 and 5 and not interferon- .[9] IL 4 and 5 are cytokines associated with the T-helper type 2 (Th2) cells whereas interferon- is associated with Th1 responses. Polarised Th2 responses are generally ineffective against tumours and viruses. Signalling via the T-cell receptor is also defective in TIL.[10]

Tumours: wounds that do not heal

Besides inflammatory cells, tumour stroma consists of new blood vessels, connective tissue, and a fibrin-gel matrix. In his 1986 review Dvorak showed how wound healing and tumour stroma formation share many important properties ("Tumors: wounds that do not heal"[11]). Wound healing is usually self-limiting whereas tumours secrete a vascular permeability factor, vascular endothelial growth factor (VEGF), that can lead to persistent extravasation of fibrin and fibronectin and continuous generation of extracellar matrix. Platelets in wounds are a critical source of cytokines, especially transforming growth factor (TGF-) and VEGF. Platelet release of such factors may also be important in tumour angiogenesis.[12] In addition, malignant cells themselves secrete proinflammatory cytokines.[13]

Proinflammatory cytokines

The cytokine network of several common tumours is rich in inflammatory cytokines, growth factors, and chemokines but generally lacks cytokines involved in specific and sustained immune responses.[13] There is now evidence that inflammatory cytokines and chemokines, which can be produced by the tumour cells and/or tumour-associated leucocytes and platelets, may contribute directly to malignant progression. Many cytokines and chemokines are inducible by hypoxia, which is a major physiological difference between tumour and normal tissue.[14] Examples are tumour necrosis factor (TNF), IL 1 and 6, and chemokines.

Tumour necrosis factor

TNF is a major mediator of inflammation, with actions directed towards both tissue destruction and recovery. While inducing death of diseased cells at the site of inflammation, TNF stimulates fibroblast growth. It can destroy blood vessels but also induce angiogenic factors.[15] Likewise, in malignant disease, high-dose local TNF selectively destroys tumour blood vessels,[16] but when chronically produced this cytokine may act as an endogenous tumour promoter, contributing to the tissue remodelling and stromal development necessary for tumour growth and spread.

TNF can be detected in malignant and/or stromal cells in human ovarian, breast, prostate, bladder, and colorectal cancer, lymphomas, and leukaemias, often in association with ILs 1 and 6 and macrophage colony stimulating factor.[13,17] In epithelial ovarian cancer, TNF mRNA is found in epithelial tumour islands, where there is a positive correlation with tumour grade.[17] The p55 TNF receptor is found on tumour and stromal cells and the p75 receptor localises to the leucocyte infiltrate in ovarian cancer, suggesting possibilities for both paracrine and autocrine action.[17] TNF is also implicated in the induction of a chemokine called monocyte chemotactic protein-1, which can regulate the macrophage and lymphocyte infiltrate,[4] and of matrix metalloprotease-9, in the ovarian tumour microenvironment. In breast cancer, infiltrating macrophages are a major source of TNF, which may regulate thymidine phosphorylase, a key angiogenic enzyme in the tumour epithelium.[18] In prostate cancer, tumour cell TNF production correlates with loss of androgen responsiveness. In non-Hodgkin lymphoma, myelogenous leukaemia, and chronic lymphocytic leukaemia, high

Glossary: **Specialised leucocytes, cytokines, chemokines**

—	Abbreviation	Group	New human nomenclature
Specialised leucocytes			
Natural killer cells	NK	. .	. .
Tumour-associated dendritic cells	TADC	. .	. .
Tumour-associated macrophages	TAM	. .	. .
Tumour-infiltrating leucocytes	TIL	. .	. .
Cytokines			
Interferon-	IFN-	Proinflammatory/Th1	. .
Interleukins 1, 6	IL-1, -6	Proinflammatory	. .
Interleukins 4, 5, 10	IL-4, -5, -10	Immune regulatory/Th2	. .
Macrophage colony-stimulating factor	M-CSF	Growth factor	. .
Migration inhibitory factor	MIF	Proinflammatory	. .
Transforming growth factor	TGF	Growth factor	. .
Tumour necrosis factor	TNF	Proinflammatory	. .
Vascular endothelial growth factor	VEGF	Angiogenic/vascular permeability	. .
Chemokines			
Eotaxin	. .	CC	CCL11
B cell attracting chemokine	BCA-1	CXC	CXCL13
Gro- /mgsa-	gro-	CXC	CXCL1
Interleukin-8	IL-8	CXC	CXCL8
IP-10	IP-10	CXC	CXCL10
Macrophage derived chemokine	MDC	CC	CCL22
Monocyte chemotactic protein-1	MCP-1	CC	CCL2
Thymus and activation regulated chemokine	TARC	CC	CCL17
Viral macrophage inhibitory protein	vMIP	CC	. .

circulating levels of TNF and its soluble receptors are associated with poor prognosis.[19]

There is also evidence for pro-cancer actions of TNF in animal models.[20–22] For example, treatment of ascitic ovarian cancer xenografts with TNF promotes adhesion of free-floating tumour cells to the peritoneum and solid tumour formation,[20] and overexpression of TNF confers invasive properties on some tumour cell lines.[21]

Direct evidence for the involvement of TNF in malignancy comes from the observation that mice lacking the gene for TNF are resistant to skin carcinogenesis.[23] TNF may be involved in the early stages of skin tumour promotion in normal mice, being transiently but extensively induced in keratinocytes after application of tumour promoter.[23] Pentoxifylline (an inhibitor of inflammatory cytokine production) inhibits papilloma development in skin carcinogenesis models,[24] and intraperitoneal injection of TNF enhances papilloma development and vascularisation of tumours.

Interleukins 1 and 6

In mouse models of metastasis, treatment with an IL-1 receptor antagonist (which inhibits the action of IL-1) significantly decreased tumour development, suggesting that local production of this cytokine aids development of metastases. Moreover, mice deficient in IL-1β were resistant to the development of experimental metastases.[25]

In human multiple myeloma the malignant cells home to the bone marrow where they stimulate stromal cells to secrete the inflammatory cytokines IL-1, IL-6, and TNF. The cytokines stimulate myeloma cell growth and promote resistance to therapy.[26] Intraperitoneal injection of mineral oil in mice induces chronic inflammation followed by myeloma. IL-6-deficient mice resist these changes, showing defective recruitment of macrophages to the peritoneum and a reduced incidence of myeloma.

Panel 2: Actions of cytokines and chemokines which may facilitate cancer growth, invasion and metastasis

- DNA damage via reactive oxygen
- Inhibition of DNA repair via reactive oxygen
- Functional inactivation of tumour suppressor genes
- Autocrine/paracrine growth and survival factors for malignant cells
- Induction of vascular permeability and extravasation of fibrin/fibronectin
- Tissue remodelling via induction/activation of matrix metalloproteinases
- Control of tumour-cell migration, direct and indirect
- Control of leucocyte infiltrate
- Modulation of cell:cell adhesion molecules
- Subversion of host immune responses
- Stimulation of angiogenesis and angiogenic factor production
- Resistance to cytotoxic drugs
- Loss of androgen responsiveness

Chemokines

Inflammatory cytokines are major inducers of a family of chemoattractant cytokines called chemokines that play a central role in leucocyte recruitment to sites of inflammation. Most tumours produce chemokines of the two major groups α (or CXC) and β (or CC).[3,27,28] Typically CXC chemokines are active on neutrophils and lymphocytes whereas CC chemokines act on several leucocyte subsets including monocytes, eosinophils, dendritic cells, lymphocytes, and natural killer cells but not neutrophils. Evidence from murine models and human tumours suggests that CC

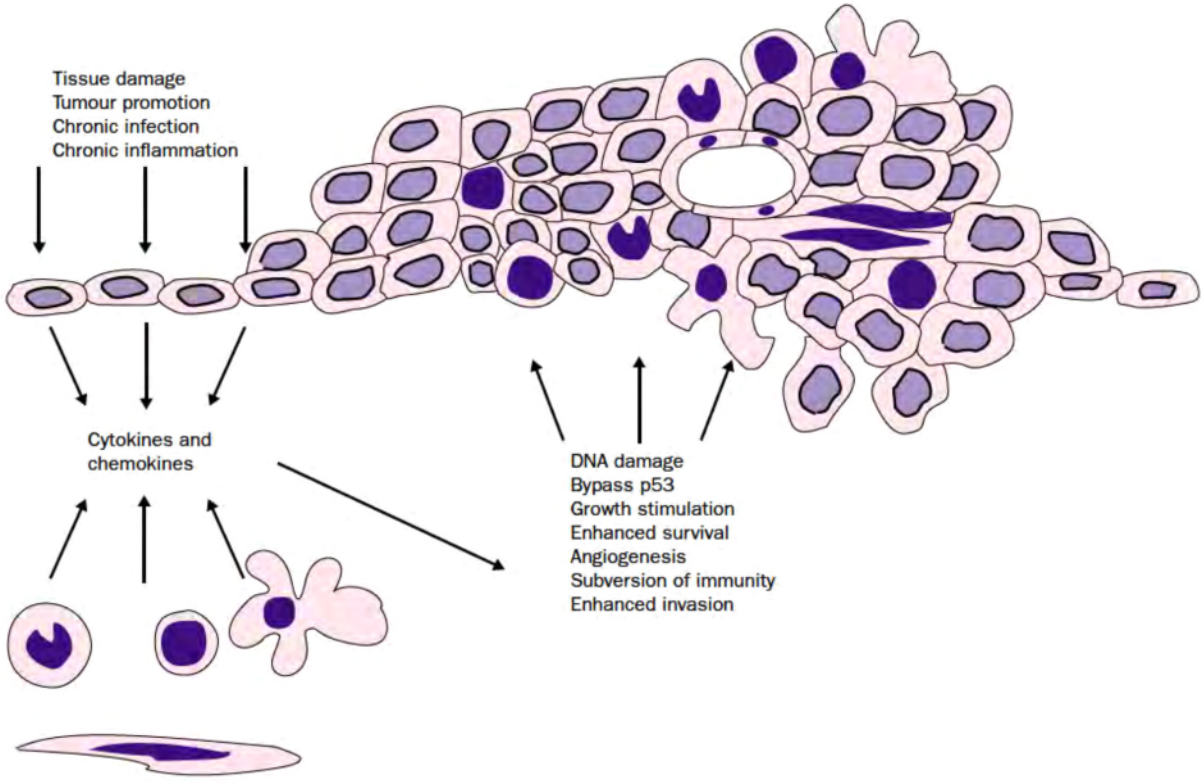

Figure 1: **Chronic inflammation, tissue damage, and chronic infection may stimulate cytokines and chemokines that contribute to development of malignant disease**

chemokines are major determinants of macrophage and lymphocyte infiltration in melanoma, carcinoma of the ovary, breast, and cervix, and in sarcomas and gliomas.[5,27,28] In Hodgkin's disease the malignant Reed-Sternberg cells express two chemokines, the macrophage-derived chemokine and thymus and activation-regulated chemokine,[9,29] that attract Th2 lymphocytes. Production of the chemokine eotaxin by stromal cells correlates with eosinophil infiltration in Hodgkin's lymphoma. Eosinophils are frequently present in tumours such as colorectal cancer.

Human and murine tumours also frequently secrete CXC chemokines such as interleukin-8. These chemokines are potent neutrophil attractants yet neutrophils are rare in tumours.[4] However, both IL-8 and a related chemokine called "gro" induce proliferation and migration of melanoma cells. When the *gro* gene was overexpressed in a non-malignant melanocyte cell line, the cells could form tumours in mice.[30] This effect probably involved both direct growth stimulation and promotion of an inflammatory response. Inflammation and wound healing have indeed been implicated in the initial steps of melanocyte oncogenesis.[31] IL-8 production is also associated with the tumorigenic and metastatic potential of pancreatic cancer cells and this chemokine is strongly inducible by hypoxia.

Helicobacter pylori induced gastritis is associated with gastric carcinoma and mucosa-associated lymphoid tissue B-cell lymphoma. BCA-1 is one of the chemokines induced by *H pylori*,[32] and it is thought that BCA-1 attracts B-cells to the mucosa where they become targets for the carcinogenic process that occurs during inflammation.

Receptors for chemokines (CCR and CXRC) are expressed both by infiltrating leucocytes and by cancer cells. The leucocytes may lose receptor expression once they are exposed to inflammatory cytokines in the tumour microenvironment, as shown for CCR2 on TAM in ovarian cancer.[33] Downregulation of CCR2 is likely to act as a signal for the retention of macrophages at the tumour site. Melanoma cells express the CXC receptors CXCR 1 and 2, and the ligand for these receptors (IL-8) will stimulate migration and proliferation of these tumour cells.[30] An ovarian cancer cell line also expressed a functional form of CXCR2.[34] These observations raise the interesting possibility that tumour cells may use chemokine gradients to spread around the body.[35]

Mechanisms of action of inflammatory cytokines in tumour microenvironment

An inflammatory cytokine network may influence survival, growth, mutation, proliferation, differentiation, and movement of both tumour and stromal cells. Moreover, these cytokines can regulate communication between tumour and stromal cells, and tumour interactions with the extracellular matrix. We will now look in more detail at the mechanisms by which cytokines and chemokines might act to promote tumours (panel 2, figure 1).

DNA damage

TNF is a transforming agent for carcinogen-treated fibroblasts. Two weeks of exposure to the cytokine in vitro is sufficient to render cells capable of tumour formation in nude mice.[36] The molecular basis may involve induction of reactive oxygen. Reactive oxygen in the form of NO is often generated by inflammatory cytokine induction of NO synthase.[37] NO can directly oxidise DNA, resulting in mutagenic changes, and may damage some DNA repair proteins.[37] Furthermore, inducible NO synthase has been detected in gynaecological carcinomas. Inflammatory cytokines may also affect genome integrity via inhibition of cytochrome p450 or glutathione S-transferase isoenzymes.

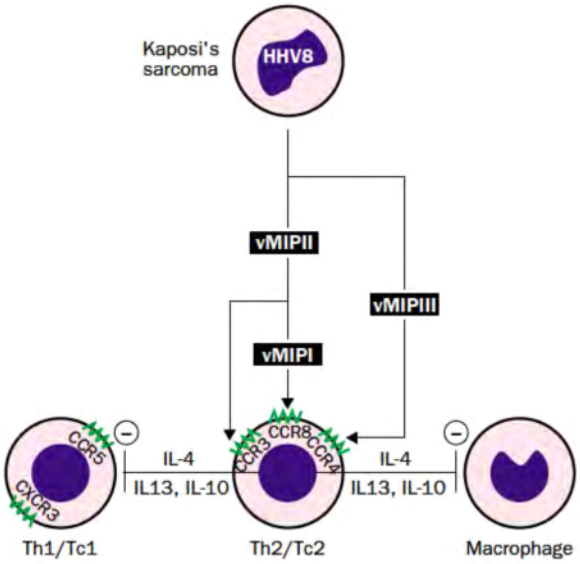

Figure 2: **Kaposi's sarcoma virus human herpesvirus 8 encodes three chemokines that recognise receptors preferentially expressed on polarised Th2 cells**
These cells are ineffective antiviral and antitumour effectors and produce cytokines which block differentiation of Th1 cells and activation of macrophages.

Bypassing p53

Another link between inflammatory cytokines and DNA damage comes from recent studies of the regulation of the tumour-suppressor protein p53. In tumours, p53 is often functionally inactivated even though the p53 gene remains intact. A search for negative regulators of p53 activity highlighted an inflammatory cytokine known as migration inhibitory factor.[38] Treatment of cells with this factor overcame p53 activity. It is not clear whether other cytokines can also inactivate p53 but chronic bypass of p53 function could enhance the proliferation of initiated cells, extend lifespan, and create a deficient response to genetic damage.[38] Migration is also strongly induced by hypoxia.[14]

Actions as growth and survival factors

Cytokines and chemokines have the potential to stimulate tumour-cell proliferation and survival and some of them may also act as autocrine growth and survival factors for malignant cells. IL-6 is a growth factor for haematological malignancies;[26] IL-1 has growth stimulating activity for gastric carcinoma that may be related to genetic predisposition[39] and for myeloid leukaemias; and growth of melanomas is promoted by IL-8 and related chemokines.[30]

Angiogenesis

Angiogenesis is important in the evolution of both cancer and inflammatory diseases that may predispose to cancer.[40] Once a tumour is established it may attain further characteristics, via mutations or hypoxia, which stimulate new blood vessels.

The inflammatory cell infiltrate, particularly TAM, may contribute to tumour angiogenesis, and there are many reports of associations between macrophage infiltration, vascularity, and prognosis.[41] Moreover TNF, IL-1, and IL-6 can stimulate production of angiogenic factors such as VEGF. Inflammatory macrophages also produce TGF-β1 that is itself angiogenic and induces production of VEGF.

Chemokines also have a role. Some CXC chemokines (eg, IL-8) are proangiogenic whereas others such as IP-10 have antiangiogenic activity.[42] Chemokines have direct

Panel 3: **Links between cancer and inflammation suggested by experimental and clinical observations**

Many inflammatory conditions predispose to cancer
Functional polymorphisms of cytokine genes associated with cancer susceptibility and severity
Distinct populations of inflammatory cells detected in many cancers
Inflammatory cytokines detected in many cancers; associated with poor prognosis, may be upregulated by local hypoxia
Chemokines detected in many cancers, associated with inflammatory infiltrate and cell motility
Deletion of cytokines and chemokines protects against carcinogens, experimental metastases and lymphoproliferative syndrome
Inflammatory cytokines implicated in action of non-genotoxic liver carcinogens
The inflammatory cytokine TNF is directly transforming in vitro

actions on microvascular endothelial cells. In addition, CC chemokines may inhibit or stimulate angiogenesis indirectly, via their influence on TAM. In many tumours (eg, non-small-cell lung cancer and pancreatic carcinoma) it is the balance between proangiogenic and antiangiogenic cytokines and chemokines, rather than absolute amounts, that regulates tumour angiogenesis.

Invasion and metastasis

Cytokines and chemokines affect various stages in the process of metastasis. TNF and CC chemokines can induce production of proteases important for invasion in both tumour cells and macrophages. Indeed, monocytes infiltrating the tumour tissue may provide cancer cells with a ready-made path for invasion (the "countercurrent invasion theory").[43] In one skin tumour model, paracrine matrix metalloproteinase-9 production by inflammatory cells was implicated in epithelial hyperproliferation, angiogenesis and increased malignant potential, and skin tumour development was reduced in mice genetically "knocked out" for this protein. Chimaeric mice expressing this metalloproteinase only in cells of bone marrow origin developed skin tumours at the same rate as control mice, highlighting the importance of stromal inflammatory cells in epithelial carcinogenesis. TNF and IL-1 augment expression of adhesion molecules on endothelial cells.[2–5] IL-18 derived from the endothelium may be the ultimate mediator of one tumour cytokine-induced adhesion molecule.[26] Certain tumour cells have receptors for adhesion molecules and use these molecular tools, typical of migrating leucocytes, to seed at distant anatomical sites.[44] Furthermore, chemokine agonists induce migration or proliferation of some tumour cells.[30] Receptors that are essential for lymphocyte and dendritic cell homing to lymph nodes,[8] could play a role in lymphatic dissemination of certain carcinomas. Direct evidence for chemokines guiding the secondary localisation of cancer has been obtained in one mouse model.[35] Mice deficient in Fas ligand develop a fatal lymphoproliferative syndrome. This phenotype is largely abolished when mice are crossed with mice unable to make TNF. One explanation may be that TNF induces chemokines that promote trafficking of the cells and accumulation of lymph nodes.[45]

Thus, tumour cells use the same molecular tools (adhesion molecules, cytokines, chemokines, chemokine receptors) and pathways as leucocytes do to spread to distant anatomical sites during inflammation.

Subversion of immunity

The prevalence of Th2 cells is common to tumours suggesting that this polarisation may be a general strategy to subvert immune responses against tumours. Inflammatory reactions are diverse, reflecting the variety of properties that can be acquired by macrophages.[46] At one extreme, interferon-activated (or type I) macrophages produce high levels of proinflammatory cytokines and Th1-attracting chemokines. At the other, activated (type II) macrophages produce high levels of antagonist to IL-1 receptor and the Th2-attracting macrophage-derived chemokine. In the murine and human tumours studied, TAM are skewed to the type II phenotype. TAM spontaneously release large amounts of IL-10 to TGF [47] both of which are immunosuppressive. Some chemokines induce IL-10 in macrophages and the monocyte chemotactic protein-1 polarises immunity in the Th2 direction.[48] Thus chronic exposure to high chemokine concentrations in the tumour microenvironment may set in motion a vicious cycle leading to skewing towards a type II inflammatory response.[47]

Some viruses encode chemokines and their inhibitors and receptors. Of particular interest is human herpesvirus type 8, which is involved in the pathogenesis of Kaposi's sarcoma. The virus genome codes for three chemokines[49] which are selective attractants of polarised Th2 cells. The virus-encoded chemokines might subvert immunity by activating type 2 responses and diverting effective Th1 defence mechanisms (figure 2).[49,50]

Interfering with chemotherapy

Another similarity between inflammation and cancer is raised plasma concentrations of acute-phase proteins (such as C-reactive protein and $_1$-acid glycoprotein). The latter binds with high affinity to, and blocks activity of, the experimental cancer drug STI571[51] which normally has activity against chronic myelogenous leukaemia in mice. If acute-phase proteins do bind to and inactivate anticancer drugs there would be obvious implications for therapy.

Local inflammaton and systemic anti-inflammation: a paradox

In terms of inflammatory reactions, neoplastic disorders constitute a paradox. Tumours produce inflammatory cytokines and chemokines and are infiltrated by leucocytes. However, neoplastic disorders are associated with a defective capacity to mount inflammatory reactions at sites other than the tumour, and circulating monocytes from cancer patients are defective in their capacity to respond to chemoattractants.[52]

Various factors originating in the tumour microenvironment may contribute to the systemic anti-inflammation associated with cancer. Chemokines leaking into the systemic circulation are likely to desensitise circulating leucocytes;[53] increased concentrations of TNF receptors and the type II decoy IL-1 receptor may buffer inflammatory cytokines; and tumours also produce anti-inflammatory cytokines.[47] Thus a defective capacity to mount a systemic inflammatory response in cancer patients could coexist with continuous leucocyte recruitment at the tumour site.

Inflammatory cytokines as cancer-modifier genes

Cytokine genes are highly polymorphic and since polymorphisms are frequently in regions of DNA that regulate transcription or posttranscriptional events, they may be functionally significant. Four studies of such polymorphisms and cancer susceptibility and severity suggest that some cytokines may be cancer-modifier genes.

Systemic release of TNF and lymphotoxin contributes to the severity of non-Hodgkin lymphoma.[19] In a study of 273 lymphoma patients, the TNF-308 polymorphism was associated with high plasma levels of the cytokine at presentation of disease.[54]

Associations have also been found between genotype changes in the promoter regions of TNF and prostate cancer. The relative risk of incidence for prostate cancer was 14-fold higher in men with the TNF-308 polymorphism and the relative incidence for prostate cancer was 17 times higher in patients with genotype *GA* at 488 region of TNF.[55]

Patients with extensive corpus gastritis, hypochlorhydria, and gastric atrophy as a result of *H pylori* infection have the greatest risk of gastric malignancy. IL-1 is upregulated during *H pylori* infection, is important in the inflammatory response of the gastric mucosa, and is a potent inhibitor of gastric acid secretion. A decreased flow of gastric secretions may increase damage by allowing accumulation of bacterial toxins and inflammatory mediators. IL-1 gene cluster polymorphisms, thought to enhance IL-1 production, confer an increased risk of chronic hypochlorhydria in response to *H pylori* and of gastric cancer.[39] Pancreatic cancer patients homozygous for allele 2 of the IL-1 gene had significantly shorter survival (144 *vs* 256 days), higher IL-1 production and higher C-reactive levels than other patients or controls.[56]

Implications for prevention and treatment

TNF blockade

Two TNF antagonists (etanercept, Enbrel [Immunex]) and infliximab, Remicade [Centocor]) have been licensed for clinical trial in the treatment of rheumatoid arthritis and Crohn's disease, with over 70 000 patients now treated.[57] There is clinical evidence for five actions of the anti-TNF antibody in rheumatoid arthritis joint tissue—namely, inhibition of cytokine/chemokine production, reduced angiogenesis, prevention of leucocyte infiltration, inhibition of matrix metalloproteases, and improvement of bone-marrow function—and all these actions would be useful in a biological therapy for cancer.

Apart from the data on TNF and cancer growth and spread, some experiments suggest a role for TNF in the development of cancer cachexia,[58] and this might be another benefit of TNF antagonist therapy. Thalidomide inhibits the processing of mRNA for TNF (and VEGF), and continuous low-dose thalidomide has shown activity in patients with advanced myeloma.[59] Several clinical studies are underway using etanercept to assess the role of anti-TNF therapy as a single agent or in combination with other therapies in malignancy. The role of etanercept in ameliorating the adverse effects of other cancer therapies is also being evaluated. There are also ongoing and planned clinical trials with infliximab. As with other "biological" approaches to cancer treatment, anti-TNF therapy may be optimal in an adjuvant setting with minimal disease. Careful recording of the incidence of malignant disease in patients receiving TNF antagonists for inflammatory disease could give some indication of the potential of these agents in the chemoprevention of cancer.

Chemokine antagonism

The chemokine system is part of the strategy used by tumours to recruit pro-tumour inflammatory responses and to seed at distinct anatomical sites. Chemokine receptors belong to a family of receptors (7 transmembrane G-protein coupled receptors) which is already a target of pharmacological interest. Tumours driven by chemokines and those where chemokines are implicated in metastasis (eg, seeding to lymph nodes) may be an appropriate target for chemokine antagonists now under development.[30,35] This approach is supported by data from mouse experiments.[60]

IL-6 antagonism

IL-6 is a major growth factor for myeloma cells.[26] In advanced disease there is an excess of IL-6 production and raised serum concentrations are associated with plasmablastic proliferative activity and short survival. When a mouse monoclonal antibody to IL-6 was given to ten patients with myeloma there was evidence of a biological effect, with decreased C-reactive protein, lower IL-6 production, and resolution of low-grade fever in six patients. However, host response to the murine antibody complicated this study.[61]

Nonsteroidal anti-inflammatory agents

People who have taken non-steroidal anti-inflammatory drugs (NSAIDs), are at reduced risk of colon cancer.[62,63] This may also be true for cancers of the oesophagus, stomach, and rectum, and in rodents experimental bladder, breast, and colon cancer is reduced when NSAIDs are administered concurrently with carcinogens.[64] NSAIDs inhibit cyclooxygenase enzymes and angiogenesis. Cyclooxygenase-2 is induced by cytokines and expressed in both inflammatory disease and cancer. When the cyclooxygenase-2 inhibitor celecoxib was tested on familial adenomatous polyposis patients in a double-blind placebo-controlled study[65] six months of twice daily treatment with 400 mg led to a significant reduction in the colorectal polyp burden.

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Exhibit 62

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Inflammation and cancer

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Abstract

Recent data have expanded the concept that inflammation is a critical component of tumour progression. Many cancers arise from sites of infection, chronic irritation and inflammation. It is now becoming clear that the tumour microenvironment, which is largely orchestrated by inflammatory cells, is an indispensable participant in the neoplastic process, fostering proliferation, survival and migration. In addition, tumour cells have co-opted some of the signalling molecules of the innate immune system, such as selectins, chemokines and their receptors for invasion, migration and metastasis. These insights are fostering new anti-inflammatory therapeutic approaches to cancer development.

The functional relationship between inflammation and cancer is not new. In 1863, Virchow hypothesized that the origin of cancer was at sites of chronic inflammation, in part based on his hypothesis that some classes of irritants, together with the tissue injury and ensuing inflammation they cause, enhance cell proliferation[1]. Although it is now clear that proliferation of cells alone does not cause cancer, sustained cell proliferation in an environment rich in inflammatory cells, growth factors, activated stroma, and DNA-damage-promoting agents, certainly potentiates and/or promotes neoplastic risk. During tissue injury associated with wounding, cell proliferation is enhanced while the tissue regenerates; proliferation and inflammation subside after the assaulting agent is removed or the repair completed. In contrast, proliferating cells that sustain DNA damage and/or mutagenic assault (for example, initiated cells) continue to proliferate in microenvironments rich in inflammatory cells and growth/survival factors that support their growth. In a sense, tumours act as wounds that fail to heal[2].

Today, the causal relationship between inflammation, innate immunity and cancer is more widely accepted; however, many of the molecular and cellular mechanisms mediating this relationship remain unresolved — these are the focus of this review. Furthermore, tumour cells may usurp key mechanisms by which inflammation interfaces with cancers, to further their colonization of the host. Although the acquired immune response to cancer is intimately related to the inflammatory response, this topic is beyond the scope of this article, but readers are referred to several excellent reviews[3,4].

An overview of inflammation

To understand the role of inflammation in the evolution of cancer, it is important to understand what inflammation is and how it contributes to physiological and pathological processes such as wound healing and infection (Fig. 1). In response to tissue injury, a multifactorial network

of chemical signals initiate and maintain a host response designed to 'heal' the afflicted tissue. This involves activation and directed migration of leukocytes (neutrophils, monocytes and eosinophils) from the venous system to sites of damage (Box 1), and tissue mast cells also have a significant role. For neutrophils, a four-step mechanism is believed to coordinate recruitment of these inflammatory cells to sites of tissue injury and to the provisional extracellular matrix (ECM) that forms a scaffolding upon which fibroblast and endothelial cells proliferate and migrate, thus providing a nidus for reconstitution of the normal microenvironment[5]. These steps involve: activation of members of the selectin family of adhesion molecules (L- P-, and E-selectin) that facilitate rolling along the vascular endothelium; triggering of signals that activate and upregulate leukocyte integrins mediated by cytokines and leukocyte-activating molecules; immobilization of neutrophils on the surface of the vascular endothelium by means of tight adhesion through $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins binding to endothelial vascular cell-adhesion molecule-1 (VCAM-1) and MadCAM-1, respectively; and transmigration through the endothelium to sites of injury, presumably facilitated by extracellular proteases, such as matrix metalloproteinases (MMPs).

Box 1

Wound healing as an example of physiological inflammation

Cellular components

Platelet activation and aggregation, in addition to accelerating coagulation, provide a bolus of secreted proteins and α-granule contents to the immediate area, all of which help initiate and accelerate the inflammatory response by the host. Examples of such secreted proteins include arachodonic acid metabolites, heparin, serotonin, thrombin, coagulation factors (factor V), adhesive proteins (fibrinogen and von Willebrand factor), plasma proteins (immunoglobulin-γ and albumin), cell growth factors (platelet-derived growth factor (PDGF), platelet-derived angiogenesis factor, transforming growth factor-α (TGF-α), TGF-β and basic fibroblast growth factor (bFGF)), enzymes (heparanase and factor XIII) and protease inhibitors (plasminogen activator inhibitor-1, α2-macroglobulin and α2-antiplasmin). Following platelet-induced haemostasis and release of TGF-β1 and PDGF, formation of granulation tissue is facilitated by chemotaxis of neutrophils, monocytes, fibroblasts and myofibroblasts, as well as by synthesis of new extracellular matrix (ECM) and neoangiogenesis.

Neutrophil chemotaxis is stimulated by factors such as circulating complement factor 5 (C5a), leukotriene B4, kallikrein, bacterial products (if present) and numerous factors released from platelets at the site (for example, PDGF, TGF-β, platelet-activating factor and platelet factor-4 (PF-4)). Although terminally differentiated with little biosynthetic machinery, neutrophils are capable of considerable production of cytokines/chemokines necessary for effector cell recruitment, activation and response15. These phagocytic cells initiate wound healing by serving as a source of early-response pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α)68, and interleukin (IL)-1α and IL-1β69. These cytokines mediate leukocyte adherence to the vascular endothelium, thus targeting and restricting leukocytes to areas of repair, and initiate repair by inducing expression of matrix metalloproteinases (MMPs) and keratinocyte growth factor (KGF/FGF-7) by fibroblasts70.

In response to tissue injury, mononuclear phagocytes (that is, macrophage progenitors) migrate from the venous system to the site of tissue injury. They are guided to the site by chemotactic factors, including PF-4, TGF-β, PDGF, chemokines (monocyte chemoattractant protein-1, -2 and -3 (MCP-1/CCL2, MCP-2/CCL8 and MCP-3/CCL7), macrophage inflammatory protein-1α and -1β (MIP-1α/CCL3 and MIP-1β/CCL4), and the cytokines IL-1β and TNF-α. Deployment of monocytes/macrophages to the site of injury

peaks as the number of neutrophils decline. Once present, however, they differentiate into mature macrophages or immature dendritic cells71. After activation, macrophages are the main source of growth factors and cytokines (TGF-β1, PDGF, bFGF, TGF-α, insulin-like growth factor (IGF)-I and -II, TNF-α and IL-1) that modulate tissue repair. Cells in their local microenvironment (for example, endothelial, epithelial, mesenchymal or neuroendocrine cells) are profoundly affected by macrophage products. Macrophages also regulate local tissue remodelling by inducing ECM components, stimulating production of proteolytic enzymes (for example, MMPs and urokinase-type plasminogen activator (uPA)), clearing apoptotic and necrotic cells, and modulating angiogenesis through local production of thrombospondin-1 (refs 72, 73).

Following their activation, mast cells are full of stored and newly synthesized inflammatory mediators. This cell type synthesizes and stores histamine, cytokines and proteases complexed to highly sulphated proteoglycans within granules, and produces lipid mediators and cytokines upon stimulation. Once activated by complement or by binding of antigens to immunoglobulin E (IgE) bound to high-affinity IgE receptors (FcεRI), they degranulate, releasing mediators including heparin, heparanase, histamine, MMPs and serine proteases, and various polypeptide growth factors, including bFGF and vascular endothelial growth factor[74]. These function both in the early initiation phase of inflammation (for example, vascular reaction and exudation), and in the late phase where leukocyte accumulation and wound healing takes place.

Chemotactic cytokines

Chemokines are classified into polypeptide groups identified by the location of cysteine residues near their amino termini (for example, C-C, C-X-C, C and CX_3C). Chemokines represent the largest family of cytokines (~41 human members), forming a complex network for the chemotactic activation of all leukocytes. Chemokine receptors, members of the seven-transmembrane-spanning G-protein-coupled receptors, vary by cell type and degree of cell activation[6]. There is considerable redundancy in chemokine-receptor interaction, as many ligands bind different receptors, or vice versa.

The composition of chemokines produced at sites of tissue wounding not only recruits downstream effector cells (as discussed above), but also dictates the natural evolution of immune reactivity. For example, MCP-1/CCL2, a potent chemotactic protein for monocytes and lymphocytes, simultaneously induces expression of lymphocyte-derived IL-4 in response to antigen challenge while decreasing expression of IL-12 (ref. 75). The net effect of this alteration facilitates a switch from a T_H1-type to a T_H2-type inflammatory response.

Tissue repair

In response to wounding, fibroblasts migrate into the wound bed and initially secrete collagen type III, which is later replaced by collagen type I. Synthesis and deposition of these collagens by fibroblasts is stimulated by factors including TGF-β1, -β2 and -β3, PDGF, IL-1α, -1β and -4, and mast cell tryptase. Once sufficient collagen has been generated, its synthesis is stopped; thus, during wound repair, production as well as the degradation of collagens is under precise spatial and temporal control.

The final phase of the healing process is re-epithelialization and migration of epithelial cells across this amalgam, in a process that requires both dissolution of the fibrin clot and degradation of the underlying dermal collagen. Epithelial cells at the leading edge of the wound express the uPA receptor, which is important for focal activation of uPA, and collagenolytic enzymes of the MMP family. In the absence of the fibrinolytic enzyme plasmin, derived from plasminogen after activation by uPA and tissue-PA, re-epithelialization is dramatically delayed[76].

The pro-inflammatory properties of TGF-β, such as leukocyte recruitment, adhesion and regulation of MMP secretion and activation, are balanced by its ability to also reverse its role, and suppress these events and foster ECM synthesis to mediate tissue repair[8]. As inflammatory cells are activated, their complement of TGF-β receptors change, resulting in differential susceptibility to TGF-β and enhanced sensitivity to suppression by TGF-β[8], a critical event to resolving inflammation.

A family of chemotactic cytokines, named chemokines, which possess a relatively high degree of specificity for chemoattraction of specific leukocyte populations[1,6,7], recruits downstream effector cells and dictates the natural evolution of the inflammatory response. The profile of cytokine/chemokines persisting at an inflammatory site is important in the development of chronic disease. The pro-inflammatory cytokine TNF-α (tumour necrosis factor-α) controls inflammatory cell populations as well as mediating many of the other aspects of the inflammatory process. TGF-β1 is also important, both positively and negatively influencing the processes of inflammation and repair[8]. The key concept is that normal inflammation — for example, inflammation associated with wound healing — is usually self-limiting; however, dysregulation of any of the converging factors can lead to abnormalities and ultimately, pathogenesis — this seems to be the case during neoplastic progression.

Neutrophils (and sometimes eosinophils) are the first recruited effectors of the acute inflammatory response. Monocytes, which differentiate into macrophages in tissues, are next to migrate to the site of tissue injury, guided by chemotactic factors. Once activated, macrophages are the main source of growth factors and cytokines, which profoundly affect endothelial, epithelial and mesenchymal cells in the local microenvironment. Mast cells are also important in acute inflammation owing to their release of stored and newly synthesized inflammatory mediators, such as histamine, cytokines and proteases complexed to highly sulphated proteoglycans, as well as lipid mediators.

Inflammation and neoplastic progression

Peyton Rous was the first to recognize that cancers develop from “subthreshold neoplastic states” caused by viral or chemical carcinogens that induce somatic changes[9,10]. These states, now known as ‘initiation’, involve DNA alterations, are irreversible and can persist in otherwise normal tissue indefinitely until the occurrence of a second type of stimulation (now referred to as ‘promotion’). Promotion can result from exposure of initiated cells to chemical irritants, such as phorbol esters, factors released at the site of wounding, partial organ resection, hormones or chronic irritation and inflammation (Fig. 1). Functionally, many promoters, whether directly or indirectly, induce cell proliferation, recruit inflammatory cells, increase production of reactive oxygen species leading to oxidative DNA damage, and reduce DNA repair. Subversion of cell death and/or repair programmes occurs in chronically inflamed tissues, thus resulting in DNA replication and proliferation of cells that have lost normal growth control. Normal inflammation is self-limiting, because the production of anti-inflammatory cytokines follows the pro-inflammatory cytokines closely (Fig. 2). However, chronic inflammation seems to be due to persistence of the initiating factors or a failure of mechanisms required for resolving the inflammatory response. Why does the inflammatory response to tumours persist?

Inflammatory cell component of tumours

Tumour cells produce various cytokines and chemokines that attract leukocytes. The inflammatory component of a developing neoplasm may include a diverse leukocyte population — for example, neutrophils, dendritic cells, macrophages, eosinophils and mast cells, as well as lymphocytes — all of which are capable of producing an assorted array of

cytokines, cytotoxic mediators including reactive oxygen species, serine and cysteine proteases, MMPs and membrane-perforating agents, and soluble mediators of cell killing, such as TNF-α, interleukins and interferons (IFNs)[11,12].

Monocytes, in the presence of granulocyte–macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-4, differentiate into immature dendritic cells[13]. Dendritic cells migrate into inflamed peripheral tissue where they capture antigens and, after maturation, migrate to lymph nodes to stimulate T-lymphocyte activation. Soluble factors such as IL-6 and CSF-1, derived from neoplastic cells, push myeloid precursors towards a macrophage-like phenotype14. Interestingly, dendritic cells found in neoplastic infiltrates are frequently immature and defective in T-cell stimulatory capacity.

Tumour-associated macrophages (TAMs) are a significant component of inflammatory infiltrates in neoplastic tissues and are derived from monocytes that are recruited largely by monocyte chemotactic protein (MCP) chemokines. TAMs have a dual role in neoplasms — although they may kill neoplastic cells following activation by IL-2, interferon and IL-12 (refs 15, 16), TAMs produce a number of potent angiogenic and lymphangiogenic growth factors, cytokines and proteases, all of which are mediators that potentiate neoplastic progression17. TAMs and tumour cells also produce IL-10, which effectively blunts the anti-tumour response by cytotoxic T cells. During development of melanoma, activated macrophages produce TGF-β, TNF-α, IL-1α, arachidonate metabolites and extracellular proteases18. In response, melanocytes express IL-8 and vascular endothelial growth factor (VEGF)-A, thereby inducing vascular angiogenesis under paracrine control[18]. Indeed, macrophage infiltration is closely associated with the depth of invasion of primary melanoma due, in part, to macrophage-regulated tumour-associated angiogenesis19.

In addition to altering the local balance of pro-angiogenic factors during melanoma development, during human cervical carcinogenesis, TAMs express VEGF-C and VEGF-D as well as the VEGF receptor-3 (VEGFR-3), all of which are implicated in formation of lymphatic vessels and lymphatic metastases[17]. By placing TAMs at the centre of the recruitment and response to angiogenic and lymphangiogenic stimuli, they may foster the spread of tumours. TAMs also induce VCAM-1 expression on mesothelial cells, a step also believed to be key for tumour cell dissemination into the peritoneum20.

The functional significance of macrophage recruitment to sites of neoplastic growth has been examined by crossing transgenic mice expressing Polyoma virus middle T (PyMT) driven by the mouse mammary tumour virus (MMTV) long terminal repeat, which are prone to development of mammary cancer, with mice containing a null mutation in the CSF-1 gene (*Csf1*^{op})[21]. Whereas the absence of CSF-1 during early neoplastic development is without apparent consequence, development of late-stage invasive carcinoma and pulmonary metastases are significantly attenuated. The key difference between PyMT mice and PyMT/*Csf1*^{op}/*Csf1*^{op} mice is not in the apparent proliferative capacity of neoplastic epithelial cells, but in the failure to recruit mature macrophages into neoplastic tissue in the absence of CSF-1. Targeting CSF-1 expression specifically to mammary epithelium in CSF-1-null/PyMT mice restores macrophage recruitment, primary tumour development and metastatic potential[12]. A similar study showed that subcutaneous growth of Lewis lung cancer cells is impaired in *Csf1*^{op}/*Csf1*^{op} mice22. In this example, however, tumours displayed a decreased mitotic index and pronounced necrosis, apparently resulting from diminished angiogenesis and impaired tumour-stroma formation. These defects were corrected by treatment of tumour-bearing mice with recombinant CSF-1 (ref. 22). Together, these genetic experiments provide a causal link between CSF-1-dependent infiltrating macrophages and the malignant potential of epithelial cells.

Macrophages are not unique among inflammatory cells in potentiation of neoplastic processes. Genetic and functional experiments indicate that neutrophils, mast cells, eosinophils and activated T lymphocytes also contribute to malignancies by releasing extracellular proteases, pro-angiogenic factors and chemokines[11,23–26].

Cancers associated with chronic inflammation

How are inflammatory cells co-opted into the neoplastic process? A plausible hypothesis is that many malignancies arise from areas of infection and inflammation, simply as part of the normal host response. Indeed, there is a growing body of evidence that many malignancies are initiated by infections[11,27–29] (Table 1) — upwards of 15% of malignancies worldwide can be attributed to infections, a global total of 1.2 million cases per year[11]. Persistent infections within the host induce chronic inflammation. Leukocytes and other phagocytic cells induce DNA damage in proliferating cells, through their generation of reactive oxygen and nitrogen species that are produced normally by these cells to fight infection[30]. These species react to form peroxynitrite, a mutagenic agent [30]. Hence, repeated tissue damage and regeneration of tissue, in the presence of highly reactive nitrogen and oxygen species released from inflammatory cells, interacts with DNA in proliferating epithelium resulting in permanent genomic alterations such as point mutations, deletions, or rearrangements. Indeed, p53 mutations are seen at frequencies similar to those in tumours in chronic inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease[31].

The strongest association of chronic inflammation with malignant diseases is in colon carcinogenesis arising in individuals with inflammatory bowel diseases, for example, chronic ulcerative colitis and Crohn's disease. Hepatitis C infection in the liver predisposes to liver carcinoma, an increased risk of bladder and colon carcinoma is associated with schistosomiasis, whereas chronic *Helicobacter pylori* infection is the world's leading cause of stomach cancer[32]. The Gram-negative bacterium *H. pylori* is established as a definite carcinogen for the development of gastric cancer — the second most common type of cancer globally11،29 — and DNA damage resulting from chronic inflammation is believed the mechanism[32]. Exacerbating DNA damage induced by inflammatory cells is expression of macrophage migration inhibitory factor (MIF) from macrophages and T lymphocytes. MIF is a potent cytokine that overcomes p53 function by suppressing its transcriptional activity[33]. Chronic bypass of p53 regulatory functions in infiltrated tissues can enhance proliferation and extend life span, while also creating an environment with a deficient response to DNA damage, amplifying accumulation of potential oncogenic mutations.

Infectious viral agents, for example, DNA tumour viruses, may also directly transform cells by inserting active oncogenes into the host genome, although other mechanisms also are responsible. While many types of infectious agents are present in animals, only a subset of individuals infected with human papilloma virus, hepatitis B virus (HBV) or Epstein-Barr virus develop virus-associated malignancies. This may reflect immune suppression, the necessity of cofactors necessary for promotion or the fact that a neoplasm can develop only if viral infection has targeted a pluripotent progenitor or stem cell. Such stem cells are typically low in abundance and located in regions of tissues protected from agents that would otherwise harm them[34]. In Rous sarcoma virus infections, inflammation is essential for tumour development and this requirement is mediated by factors such as TGF-β and other cytokines produced by the inflammatory cells35. Epstein-Barr virus also causes sustained proliferation of B lymphocytes, which, when coupled with a secondary mutation, can result in neoplastic progression and malignant conversion to give rise to Burkett's lymphoma.

The molecular mechanism behind the associated risk of hepatocellular carcinoma resulting from HBV and/or hepatitis C virus (HCV) infection is uncertain. Although there is evidence for clonal integration of viral DNA in tumours and surrounding parenchyma cells, there are no

defined transforming sequences found within the viral genomes that can act as viral oncogenes. Moreover, there is no evidence to suggest that viral integration activates either a classical cellular oncogene or inactivates a cellular tumour suppressor gene. HCV core protein interacts with the signal transducer and activator of transcription 3 (STAT3) protein[36], a transcription factor involved in mediating cytokine signalling37. This interaction induces sustained phosphorylation of a critical tyrosine residue, resulting in enhanced proliferation and upregulation of Bcl-x_L and cyclin-D. Thus, chronic viral replication in hepatocytes may alter the local cytokine profile and the apoptotic or proliferative responses in infected cells, with an immune response to the viral proteins resulting in a state of chronic inflammation. Interestingly, a similar pathway involving inflammation, IL-6 and STAT3 is downstream of *H. pylori* in the generation of stomach cancer[38].

The chemokine connection

Chemokines were initially defined functionally as soluble factors regulating directional migration of leukocytes during states of inflammation; however, chemokine biology extends to all cell types, including most human neoplastic cells[6]. Attention first focused on the role of chemokines during malignancy when it was reported that experimental animals without T or natural killer (NK) cell functions, when challenged with a tumour, showed a typical inflammatory infiltrate; this suggested that neoplastic cells either produce chemotactic factors or induce their expression in nearby 'host' cells[39]. It is now appreciated that the chemokine-receptor system can be altered dramatically in neoplastic tissue, particularly at the invasive edges. Moreover, chemokines induce direct effects on stromal and neoplastic cells in addition to their roles in regulating leukocyte recruitment (Fig. 2).

Regulation of tumour growth by chemokines—Some tumour cells not only regulate their chemokine expression to help recruit inflammatory cells, but also use these factors to further the tumour growth and progression. Melanoma is perhaps the best exemplar in which chemokines (for example, GROα/CXCL1, GROβ/CXCL2, GROγ/CXCL3 and IL-8/CXCL8) have been shown to exert autocrine control over neoplastic cell proliferation[40]. Blocking GROα or the CXCR2 receptor attenuates melanoma cell proliferation *in vitro*[41], whereas overexpression of GROα, GROβ or GROγ in a variety of tumour-derived cell lines enhances their colony-forming activity and tumorigenicity in nude mice[42,43]. Other CXCR2 ligands have been identified as having autocrine roles in the growth of pancreatic, head and neck, and non-small-cell lung carcinoma[44,45], whereas in mouse models, ENA-78/CXCL5 variably affects tumour growth, vascularity and apoptosis[46]. Macrophage pro-inflammatory chemokine-3α (MIP-3α/CCL20), a CC chemokine, is overexpressed in pancreatic carcinoma cells and infiltrating macrophages adjacent to tumours; MIP-3α/CCL20 stimulates growth of neoplastic cells while simultaneously enhancing migration of TAMs[47].

Regulation of angiogenesis by chemokines—Activation of angiogenic programmes represents a shift in the balance between pro- and anti-angiogenic factors48. Although angiogenesis is strictly controlled, it is associated with chronic inflammatory diseases, such as psoriasis, rheumatoid arthritis and fibrosis, as well as with tumour growth and metastasis[48]. It is well established that CXC chemokines with the three amino acids (Glu-Leu-Arg/ELR) immediately amino-terminal to the CXC motif (ELR^+) are pro-angiogenic and stimulate endothelial cell chemotaxis, whereas ELR^- CXC chemokines (for example, PF-4/CXCL4, MIG/CXCL9 and IP-10/CXCL10) possess angiostatic activities[44,49]. ELR^+ CXC ligands bind to CXCR2 and to a lesser degree to CXCR1, whereas ELR^- CXC ligands bind to CXCR3, CXCR4 and CXCR5 (ref. 6). Compared to VEGF-A, murine MCP-5/CCL12 exhibits only modest mitogenic properties towards endothelial cells; however, it is a potent chemoattractant. In contrast, stromal-cell-derived factor 1 (SDF-1/CXCL12) induces endothelial expression of VEGF-A; VEGF-A in turn upregulates CXCR4 on endothelial cells7. Although it is not always

clear if the angiostatic and angiogenic effects of chemokines are direct or indirect, it is accepted that the balance between the two regulates neoplastic cell physiology.

Chemokines and metastasis—Malignant cells that possess metastatic capacity have properties endowing them with the ability to invade and survive in ectopic tissue, venous and/or lymphatic environments, as well as ability to reside and proliferate at a distal site (Fig. 3). Much debate exists as to whether malignant cells metastasize to environments favouring their specific growth or whether different organs are endowed with the ability to arrest or attract specific types of malignant cells through chemotactic factors (the so-called homing theory) 48. Studies using a mouse model by Muller and colleagues suggest that the pattern of breast cancer metastases is in part governed by specific interactions between CXCR4 and its ligand SDF-1/CXCL12 (ref. 50). CXCL12 is a rather unique chemokine in that it is the product of resting cells in multiple organs6, and is particularly highly expressed in target organs for breast cancer metastasis50. CXCL12 triggers chemotaxis of malignant mammary carcinoma cells *in vitro*, and the chemotactic activity of extracts of organs targeted by breast cancer cells (bone marrow, liver, lung and lymph nodes) can be neutralized by anti-CXCR4 antibodies. The involvement of CXCR4 in metastasis is not limited to breast cancer, as CXCR4 is expressed in tumour cell lines (for example, prostate carcinomas, B-cell lymphomas, astrogliomas and chronic lymphocytic leukaemias) that also respond to CXCL12 (ref. 51). The broader implications of these observations are that chemokines may be involved in regulating the spectrum of metastases in diverse cancer types.

Tumours commandeer leukocyte adhesion mechanisms

Tumour cells not only take advantage of the trophic factors made by inflammatory cells, but may also use the same adhesion molecules, chemokines and receptors to aid in migration and homing during distant metastatic spread. Evidence suggests that mechanisms used for homing of leukocytes may be appropriated for the dissemination of tumours via the bloodstream and lymphatics. Selectins are adhesion receptors that normally recognize certain vascular mucin-type glycoproteins bearing the carbohydrate structure sialyl-Lewis X and facilitate leukocyte rolling along the blood vessels. Metastatic progression of many epithelial carcinomas correlates with tumour production of mucins containing sialyl-Lewis X. Lung colonization by melanoma cells that express sialyl-Lewis X is significantly reduced in E/P-selectin-deficient mice[52]. P-selectin deficiency attenuates tumour growth and metastasis, and tumours are significantly smaller in mice treated with a receptor antagonist peptide.

These results indicate that receptors expressed in the vasculature are crucial in targeting sialyl-Lewis X-dependent cancer cells[53]. P-selectin facilitates human carcinoma metastasis in immunodeficient mice by mediating early interactions of platelets with blood-borne tumour cells via their cell-surface mucins, a process that can be blocked by heparin[54]. L-selectin on neutrophils, monocytes and/or NK cells also may facilitate metastasis[55]. Metastasis could involve the formation of tumour–platelet–leukocyte emboli that interact with the vasculature of distant organs. In addition, expression of L-selectin on tumour cells can foster metastasis to lymph nodes[56].

Inflammation as an anti-cancer therapeutic opportunity

Perhaps the best evidence for the significance of inflammation during neoplastic progression comes from study of cancer risk among long-term users of aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs). Much data indicates that use of these drugs reduces colon cancer risk by 40–50%, and may be preventative for lung, oesophagus and stomach cancer57,58. The ability of NSAIDs to inhibit cyclo-oxygenases (COX-1 and -2) underlies their mechanism(s) of chemoprevention. COX-2 converts arachidonic acid to prostaglandins, which in turn induces inflammatory reactions in damaged tissues59. Aspirin is non-selective

in its inhibition of platelet function by acetylating and irreversibly inactivating both COX-1 and COX-2. Inactivation prevents platelet synthesis of prostaglandins, endoperoxides and thromboxane A2.

Other NSAIDs, for example, flurbiprofen, may have strong anti-metastatic effects because of their inhibition of platelet aggregation[60]. But NSAIDs may act through mechanisms other than inhibition of COX enzyme activity alone, as some NSAIDs lacking COX-inhibitory function show efficacy in inhibiting colon carcinogenesis[61]. Other mechanisms have been proposed[15], including induction of apoptosis through release of cytochrome C from mitochondria and subsequent activation of caspase-9 and -3, and/or interference with cell-cycle progression, reduction of carcinogen activation and stimulation of immune surveillance.

The pro-inflammatory cytokine TNF-α is also a key downstream mediator in inflammation. Despite the name, TNF-α is important in early events in tumours, regulating a cascade of cytokines, chemokines, adhesions, MMPs and pro-angiogenic activities[1,62]. Thus, TNF-α may be one of the ways in which inflammation acts as a tumour promoter. Blocking antibodies that have significant therapeutic efficacy in other inflammatory diseases[63] may have applications in therapy in cancer.

Tumours are also rich in mucins and other ligands that may include the sialyl-Lewis X epitope recognized by selectins. Because selectins may have a role in metastasis[54,55], targeting the selectin interaction with heparin or antagonists of the receptor may decrease metastasis[54].

MMPs are produced by inflammatory cells and by stromal cells responding to chemokines and cytokines produced by inflammatory cells in tumour microenvironments[25]. Like inflammatory cells, MMPs may both promote tumour progression and attenuate it. Indeed, MMPs may mediate many of the actions of inflammatory cells in neoplasms[64]. MMPs can recruit inflammatory cells by releasing chemoattractants and motogens; they also generate growth-promoting and cytostatic signals. MMPs activate angiogenesis, but also produce fragments of basement-membrane collagens and plasminogen that are angiogenesis inhibitors. They have both apoptotic and anti-apoptotic actions. Thus, the efficacy of MMP inhibitors may be mediated, at least in part, through anti-inflammatory actions[64,65]. Given their diverse actions, it is also not surprising that trials with MMP inhibitors have had mixed results, with efficacy reported mostly during early tumour progression[66].

Inflammatory cells and cancer: friend or foe?

It is now evident that inflammatory cells have powerful effects on tumour development. Early in the neoplastic process, these cells are powerful tumour promoters, producing an attractive environment for tumour growth, facilitating genomic instability and promoting angiogenesis. The inflammatory cells, and the chemokines and cytokines that they produce, influence the whole tumour organ, regulating the growth, migration and differentiation of all cell types in the tumour microenvironment, including neoplastic cells, fibroblasts and endothelial cells. Later in the tumorigenic process, neoplastic cells also divert inflammatory mechanisms such as selectin–ligand interactions, MMP production and chemokine functions to favour neoplastic spread and metastasis. This may be part of an attempt by the tumour to subvert immune cell functions, so favouring tumour development. Yet, the recruitment of inflammatory cells may also be counterproductive for tumour development, and also may represent an attempt by the host to suppress tumour growth.

The pro-tumour actions of inflammatory cells include releasing growth and survival factors, promoting angiogenesis and lymphangiogenesis, stimulating DNA damage, remodelling the ECM to facilitate invasion, coating tumour cells to make available receptors for disseminating cells via lymphatics and capillaries, and evading host defence mechanisms. Although

inflammatory responses should also be anti-tumour, cancer patients are often defective in their inflammatory responses. This may arise by two distinct tumour-mediated mechanisms: a failure to upregulate the anti-inflammatory cytokines, or subversion of the host response resulting from desensitization of receptors owing to high chemokine and cytokine concentrations that then blunt systemic responses. Can we apply these new insights for targeting metastases?

It is clear that anti-inflammatory therapy is efficacious towards early neoplastic progression and malignant conversion. In a fully developed malignancy, there are 'excess' inflammatory cells in the tumour microenvironment. Does the tumour need inflammation to help foster angiogenesis? We must think globally and act locally. One approach is to evaluate whether functional polymorphisms in genes that regulate inflammatory processes (for example, genes encoding MMPs, cytokines, chemokines or selectins) harbour altered risk for developing cancer or are indicators of prognosis. Yet for all the local inflammation in tumours, in many cases the overall innate immunity of the host is blunted. The challenge for the future is to normalize the inflammatory network to regain a normal host response overall: decreasing the high levels of tumour-promoting properties of the infiltrating cells, such as pro-inflammatory cytokines, while increasing their tumour-suppressing properties, such as anti-inflammatory cytokines. In this way, later in tumour progression, we can harness the activities that are anti-tumour while suppressing those that are pro-tumour.

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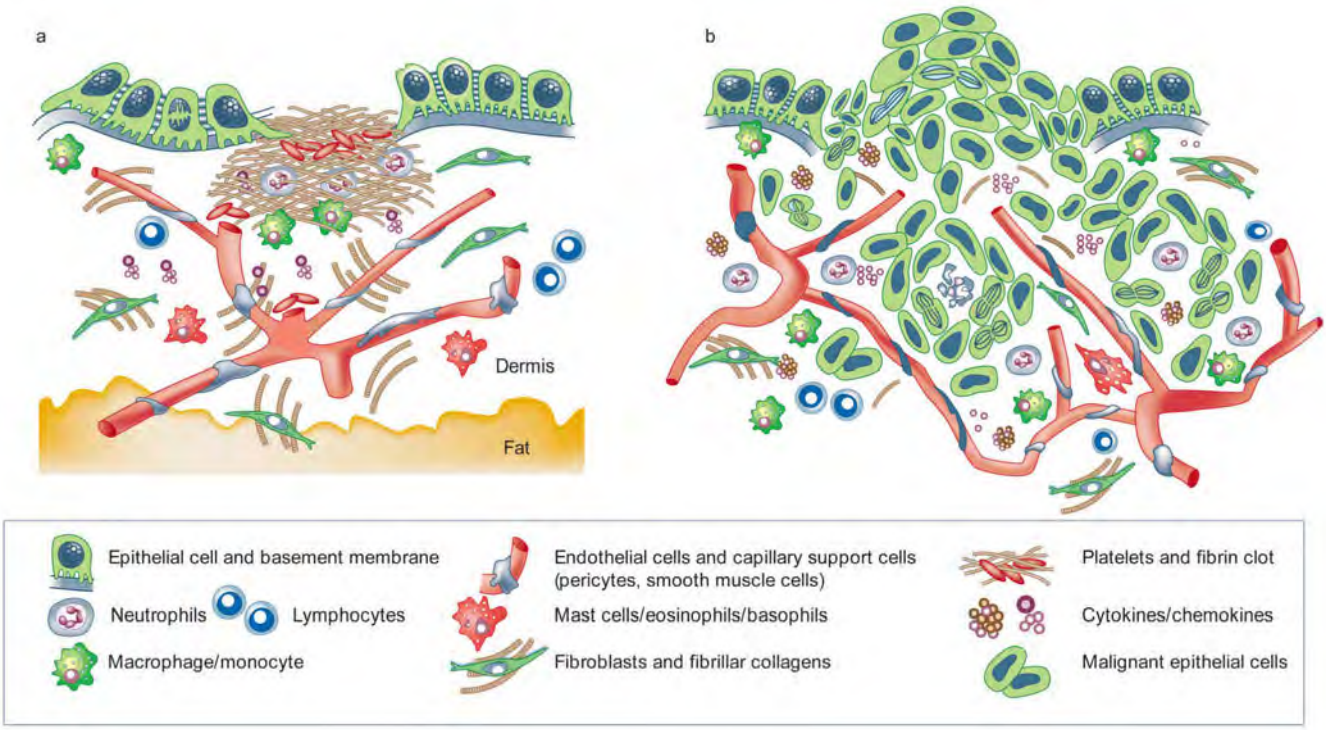

Figure 1.

Wound healing versus invasive tumour growth. **a**, Normal tissues have a highly organized and segregated architecture. Epithelial cells sit atop a basement membrane separated from the vascularized stromal (dermis) compartment. Upon wounding or tissue assault, platelets are activated and form a haemostatic plug where they release vasoactive mediators that regulate vascular permeability, influx of serum fibrinogen, and formation of the fibrin clot. Chemotactic factors such as transforming growth factor-β and platelet-derived growth factor, derived from activated platelets, initiate granulation tissue formation, activation of fibroblasts, and induction and activation of proteolytic enzymes necessary for remodelling of the extracellular matrix (for example, matrix metalloproteinases and urokinase-type plasminogen activator). In combination, granulocytes, monocytes and fibroblasts are recruited, the venous network restored, and re-epithelialization across the wound occurs. Epithelial and stromal cell types engage in a reciprocal signalling dialogue to facilitate healing. Once the wound is healed, the reciprocal signalling subsides. **b**, Invasive carcinomas are less organized. Neoplasia-associated angiogenesis and lymphangiogenesis produces a chaotic vascular organization of blood vessels and lymphatics where neoplastic cells interact with other cell types (mesenchymal, haematopoietic and lymphoid) and a remodelled extracellular matrix. Although the vascular network is not disrupted in the same way during neoplastic progression as it is during wounding, many reciprocal interactions occur in parallel. Neoplastic cells produce an array of cytokines and chemokines that are mitogenic and/or chemoattractants for granulocytes, mast cells, monocytes/macrophages, fibroblasts and endothelial cells. In addition, activated fibroblasts and infiltrating inflammatory cells secrete proteolytic enzymes, cytokines and chemokines, which are mitogenic for neoplastic cells, as well as endothelial cells involved in neoangiogenesis and lymphangiogenesis. These factors potentiate tumour growth, stimulate angiogenesis, induce fibroblast migration and maturation, and enable metastatic spread via engagement with either the venous or lymphatic networks.

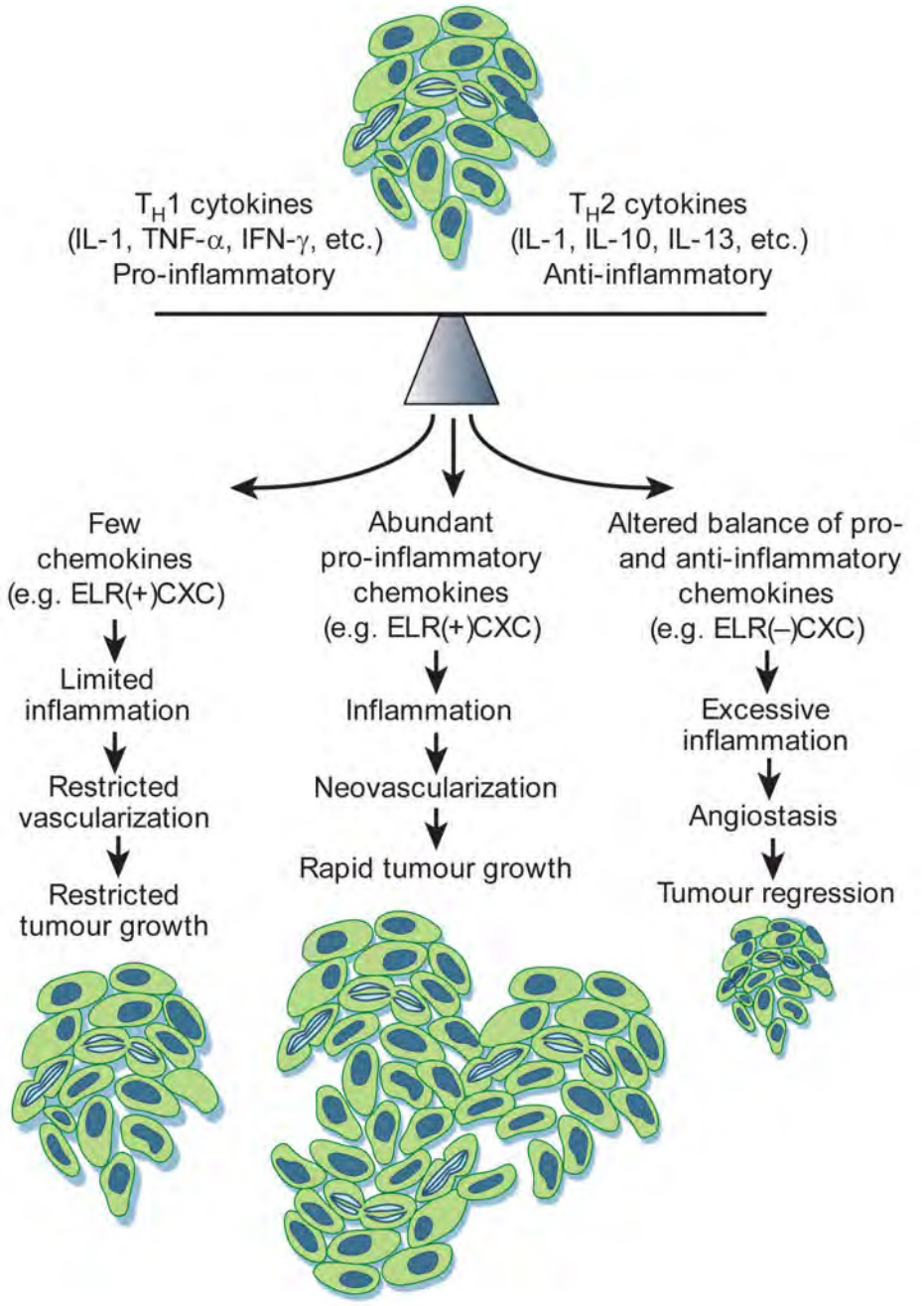

Figure 2.

Cytokine and chemokine balances regulate neoplastic outcome. The balance of cytokines in any given tumour is critical for regulating the type and extent of inflammatory infiltrate that forms. Tumours that produce little or no cytokines or an overabundance of anti-inflammatory cytokines induce limited inflammatory and vascular responses, resulting in constrained tumour growth. In contrast, production of an abundance of pro-inflammatory cytokines can lead to a level of inflammation that potentiates angiogenesis, thus favouring neoplastic growth. Alternatively, high levels of monocytes and/or neutrophil infiltration, in response to an altered balance of pro-versus anti-inflammatory cytokines, can be associated with cytotoxicity,

angiostasis and tumour regression. In tumours, interleukin-10 is generally a product of tumour cells and tumour-associated macrophages.

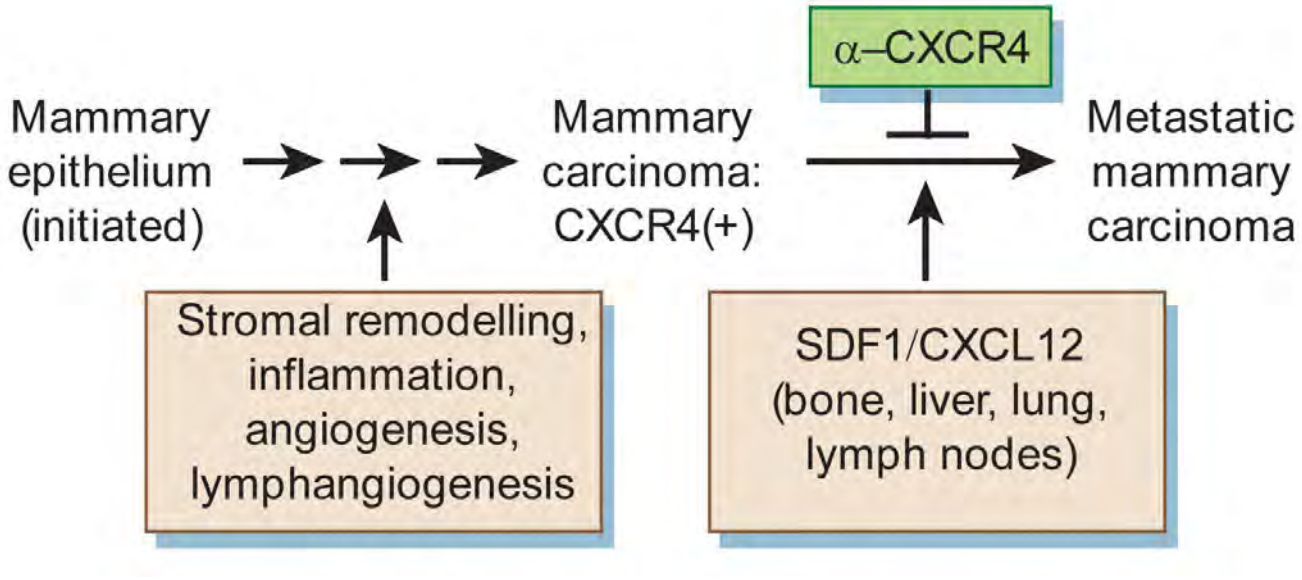

Figure 3.

Cancer metastasis and chemokine signalling. Initiated epithelial cells are promoted by inflammation to undergo neoplastic progression, a process that requires remodelling of the extracellular matrix, recruitment of inflammatory cells, angiogenesis and lymphangiogenesis. Out of this microenvironment, carcinomas arise. These neoplastic cells then turn on expression of chemokine receptors, such as CXCR4. The production of chemokine ligands for these receptors, in sites such as lymph nodes, bone marrow, liver and lung, then facilitates their invasion and migration to secondary sites where malignant cells reside either in a dormant state, or proliferate to form a productive metastatic lesion. Blockade of chemokine receptors, for example, anti-CXCR4 antibodies, attenuates metastatic spread in some experimental systems.

Table 1

Chronic inflammatory conditions associated with neoplasms

Pathologic condition	Associated neoplasm(s)	Aetiologic agent
Asbestosis, silicosis	Mesothelioma, lung carcinoma	Asbestos fibres, silica particles
Bronchitis	Lung carcinoma	Silica, asbestos, smoking (nitrosamines, peroxides)
Cystitis, bladder inflammation	Bladder carcinoma	Chronic indwelling, urinary catheters
Gingivitis, lichen planus	Oral squamous cell carcinoma	
Inflammatory bowel disease, Crohn's disease, chronic ulcerative colitis	Colorectal carcinoma	
Lichen sclerosus	Vulvar squamous cell carcinoma	
Chronic pancreatitis, hereditary pancreatitis	Pancreatic carcinoma	Alcholism, mutation in trypsinogen gene on Ch. 7
Reflux oesophagitis, Barrett's oesophagus	Oesophageal carcinoma	Gastric acids
Sialadenitis	Salivary gland carcinoma	
Sjögren syndrome, Hashimoto's thyroiditis	MALT lymphoma	
Skin inflammation	Melanoma	Ultraviolet light
Cancers associated with infectious agents		
Opisthorchis, *Cholangitis*	Cholangiosarcoma, colon carcnoma	Liver flukes (*Opisthorchis viverrini*), bile acids
Chronic cholecystitis	Gall bladder cancer	Bacteria, gall bladder stones
Gastritis/ulcers	Gastric adenocarcinoma, MALT	*Helicobacter pylori*
Hepatitis	Hepatocellular carcinoma	Hepatitis B and/or C virus
Mononucleosis	B-cell non-Hodgkin's lymphoma, Burkitts lymphoma,	Epstein-Barr Virus
AIDS	Non-Hodgkin's lymphoma, squamous cell carcinomas, Kaposi's sarcoma	Human immunodeficiency virus, human herpesvirus type 8
Osteomyelitis	Skin carcinoma in draining sinuses	Bacterial infection
Pelvic inflammatory disease, chronic cervicitis	Ovarian carcinoma, cervical/anal carcinoma	Gonnorrhoea, chlamydia, human papillomavirus
Chronic cystitis	Bladder, liver, rectal carcinoma, follicular lymphoma of the spleen	Schistosomiasis

Modified from refs 29, 67. MALT, mucosa-associated lymphoid tissue.